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Unveiling the antimalarial properties of *Terminalia ivorensis* (A. Chev) stem bark aqueous extract: In vivo efficacy testing and in silico predictions

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ARTICLE INFO

ABSTRACT

Due to the spreading resistance to antimalarial drugs, new therapeutics are urgently needed, preferably with novel modes of action. Extracts from *Terminalia ivorensis* have previously been shown to possess activity in vitro against multidrug-resistant and drug-sensitive strains of *Plasmodium falciparum*. However, to the best of our knowledge, no scientific study has been published describing the antimalarial potential of these extracts through in vivo efficacy testing. This study aimed to determine the safety and antimalarial efficacy of the *T. ivorensis* stem bark aqueous extract (TiH$_2$O) in a mouse model using the OECD 423 protocol and the suppressive and curative murine malaria models, and to predict in silico the pharmacokinetic properties and drug-likeness of two major phytochemical constituents. The in vivo antimalarial efficacy was assessed using the *P. berghei* NK65-infected mice. The TiH$_2$O treatment impact on biochemical parameters was measured using established standard procedures. The pharmacokinetics prediction was achieved through the pkCSM predictor and Swiss ADME. The TiH$_2$O extract was nontoxic in BALB/c mice at a lethal dose of 50 (LD$_{50}$) > 2000 mg/kg. The TiH$_2$O extract displayed strong antimalarial efficacy with 100% parasitemia suppression at 200 mg/kg b.w. after 4 days of treatment while its oral administration at 400 mg/kg b.w. in the curative model significantly decreased *P. berghei* parasitemia by 94.07% with a median efficacy dose (ED$_{50}$) of 96.80 mg/kg. The administration of TiH$_2$O extract restored the histological parameters disrupted by *P. berghei*, and the transaminase (ALT and AST) activity, creatinine, and bilirubin levels significantly decreased compared to the negative control mice. In silico explorations showed that the main constituents leucodelphidin (leucodelphinidin) and ellagic acid of the TiH$_2$O extract have drug-like properties, thus indicating that *T. ivorensis* might constitute a promising source of antimalarial chemical entities with good pharmacokinetics and drug-like properties. The results obtained further corroborated the preliminary in vitro antiplasmodial studies of *T. ivorensis* stem bark aqueous extract. The metabolome of TiH$_2$O extract should be further profiled in the prospects of characterizing novel natural product scaffolds to support antimalarial drug discovery.

1. Introduction

Malaria, a life-threatening disease caused by *Plasmodium* parasites, continues to pose a significant global health burden (Sumbe & Barkade, 2023). The most severe form and treatment failure as well as the highest prevalence of malaria are attributed to *P. falciparum* and *P. vivax* (Sumbe & Barkade, 2023; World Malaria Report, 2023). The World Health Organization estimated an increase in malaria cases in 2022 with 249 million cases compared to 244 million cases in 2021 with Africa accounting for over 94% of all malaria cases and 95% (580 000) of deaths (World Malaria Report, 2023). The majority of malaria-related deaths in Africa (~ 80%) occur in children under the age of five, and the deadliest *P. falciparum* elicits the heaviest burden (Sumbe & Barkade, 2023). *Plasmodium* parasites spread to humans through mosquito bites and quickly enter the bloodstream, penetrate hepatocytes, and reproduce asexually, creating thousands of merozoites (Luth et al., 2018). In *P. vivax* and *P. ovale*, sporozoites may mature into "hypnozoites", a latent form that can revive weeks or months later and cause an infection recurrence. The liver exoerythrocytic cycle replicates asexually and thrives in the intraerythrocytic stage. The disease's symptomatic phase is brought on by merozoites that are discharged after leaving the lysed host red blood cells. After several cycles of replication, a small subset of parasites diverts from asexual replication and instead produce sexual progeny that differentiates into male and female sexual forms, called gametocytes that can subsequently be transmitted into the mosquito to perpetuate the infection cycle (Luth et al., 2018; Sumbe & Barkade, 2023). *P. falciparum* is not only the most deadly and common malaria parasite but also the hardest to eradicate, as it perpetually finds a means to evade every drug in the pharmaceutical arsenal (Rocamora & Winzeler, 2020).

Despite achievements with artemisinin-based combination treatments (ACTs), eradication efforts still face numerous obstacles, particularly in the African region (World Malaria Report, 2022) due to the recent emergence of artemisinin-resistant parasites (Asua et al., 2021; Mihreteab et al., 2023; Uwimana et al., 2020; Uwimana et al., 2021). This trend in the African context is of higher concern. In that regard, novel drugs are critically needed to help control malaria. The plant origin of famous antimalarial drugs such as quinine isolated from the bark of *Cinchona* trees and artemisinin isolated from *Artemisia annua* sustains the hope that continuous consideration of biodiversity could unveil novel starting points for new drug development against malaria. In this line, the investigation of natural products to identify novel pharmacophores with acceptable profiles as starting points for antimalarial drug discovery is a credible ongoing approach.

Terminalia ivorensis A. Chev (Combretaceae), also known as idigbo, black afara, shingle wood, brimstone, and black bark, is extensively used in traditional medicines around the world to treat a variety of illnesses, including malaria and yellow fever (Eloff et al., 2008). Of note, previous studies have indicated the potency of *T. ivorensis* against asexual blood stages of drug-sensitive and drug-resistant malaria parasites (Annan et al., 2012; Appiah-Opong et al., 2022; Komlaga et al., 2016). We have recently reported the antiplasmodial potency of this plant in vitro (Tali et al., 2022). However, the exploration of these natural product extracts using in vivo malaria models has not been reported yet.

The present study aimed to decipher the safety and efficacy properties of the aqueous extract from *T. ivorensis* (TiH$_2$O) and to predict the pharmacokinetic properties and drug-likeness of two of its active phytochemicals.

2. Materials and methods

2.1. Ethical approval

Animal experiments were performed following the protocol approved by the Joint Institutional Review Board, University of Yaounde 1 (No. BTC-JIRB2023-091), and the results were reported following the ARRIVE guidelines (S1 Checklist).

2.2. Plant materials

T. ivorensis (commonly referred to as "black afara" in Cameroon) was identified at Carrefour MEEC-Nkolbisson, Yaounde-Cameroon by Mr. Victor Nana, a taxonomist from the National Herbarium of Cameroon, Yaounde. Sample from the stem bark was collected around 6.30 am local time and a voucher specimen was deposited at the Cameroon National Herbarium under reference number 48878/HNC.

2.3. Extraction of plant material

The stem bark of *T. ivorensis* was ground into fine powders (∅~0.5 µm) using a miller (Hammer Mill, Leabon 9FQ, Zhengzhou, PRC) and dried under shade at room temperature (25–29 °C) for two weeks (Tali et al., 2022). 1.5 kg of powder was macerated in 4.5 l of distilled water for three consecutive days. The macerate was then filtered using Whatman No 1 filter paper, and the residue was macerated afresh for two consecutive days and treated similarly. The extract was lyophilized using a Virtis Wizard 2.0 Freeze Dryer Lyophilizer (Model: XLS-70) (Tali et al., 2022). The extraction yields were determined relative to the initial weight of the powder, and the dried crude extract (greenish-brown color) was used for in vivo safety and efficacy studies.

2.4. Determination of the oral acute toxicity of TiH2O extract

The potential acute toxicity of TiH$_2$O extract was evaluated in mice as previously described (OECD, 2002). Three groups of three mice each were used for this study. Group one was orally administered by gavage as previously described (Anh Thu Pham et al., 2011) with a single dose (2000 mg/kg b.w.) of TiH$_2$O extract while group two (control group) was treated with 20 ml/kg distilled water. Group three was considered as the normal group and received no extract or water. The animals were observed for 30 minutes, 4 hours, and thereafter daily for 14 days to record any signs of toxicity.

2.5. Determination of bioactivity parameters in the murine experimental model

2.5.1. Amplification and maintenance of P. berghei NK65 in mice

A suspension of *P. berghei*-infected erythrocytes in PBS (1:1 v/v) was injected into the peritoneal cavity of healthy BALB/c mice. Parasite proliferation was monitored microscopically using Giemsa-stained slides, and when the parasitemia reached 10-20%, mice were sedated with a KX cocktail (ketamine/xylazine 120/16 mg/kg) (Sloan et al., 2011). For parasite amplification and maintenance, blood was collected by jugular puncture into EDTA tubes and diluted in PBS before inoculation to another group of healthy mice.

2.5.2. Determination of the antimalarial activity of TiH_2O

2.5.2.1. Assessment of the suppressive activity of TiH_2O

Parasitemia suppression by TiH_2O was assessed according to the method described by Knight and Peters (1980) using early *P. berghei*-infected mice. Chloroquine was used as a positive control (Knight & Peters, 1980). Treatment was administered by oral gavage to mimic the traditional route of administration. Three treatment groups of six mice each including one test group receiving TiH_2O, one positive control group receiving chloroquine (CQ), and one negative control group receiving distilled water were included. On day 1 of post-infection (D0), whole blood was drawn from the donor mouse by jugular puncture into an EDTA tube, and a suspension of *P. berghei*-parasitized erythrocytes in PBS was prepared at 1×10^6 RBCs/ml. Then, the experimental mice were infected intraperitoneally with 200 µl of the so-called suspension. Two hours after infestation, mice were treated orally with 100 mg/kg b.w. of TiH_2O. Positive and negative control mice received 10 mg/kg b.w. chloroquine and 25 ml/kg b.w. distilled water, respectively. All animal groups were thereafter monitored similarly for 4 consecutive days (D0–D3) between 8 a.m. and 10 a.m. with daily recording of parasitemia and survival rates. To evaluate the ability of TiH_2O to prevent weight loss due to infection, weight differences between days post-infestation were calculated relative to D0. Every 48 hours, Giemsa-stained thin smears were prepared from tail blood from the 5th to the 15th day (D4–D14) and examined microscopically at x100 magnification to determine the percent parasitemia relative to the total red blood cell (RBC) counts.

The average percentage of chemo-suppression was calculated for the treated groups as given below:

$$Chemosupression\ (\%) = \frac{A - B}{B} \times 100$$

where A and B represent the average percent parasitemia in the negative control group and the test group respectively. Survival rate was monitored twice daily and was determined over 14 days (D0–D13) and compared between groups.

2.5.2.2. Assessment of the curative effect of TiH_2O

The assessment of the therapeutic activity of TiH_2O was conducted as previously described (Knight & Peters, 1980). On day 1, 30 healthy mice were intraperitoneally injected with 200 µl of *P. berghei* NK65-infected RBCs. After illness induction three days later corroborated by established parasitemia (3 to 4%), five groups of six mice each receiving daily oral doses of 100, 200, and 400 mg/kg/b.w. of TiH_2O (groups 1, 2, and 3, respectively); 10 mg/kg/b.w. of CQ (group 4); and 10 ml/kg distilled water (group 5) were considered. Giemsa-stained thin blood smears were examined daily to monitor parasitemia changes in response to drug exposure. Treatments were repeated for five consecutive days and the data were normalized to percent control activity using Microsoft Excel software, and median efficacy dose (ED_{50}) for TiH_2O was calculated using Prism 8.0 software (GraphPad) with data fitted by nonlinear regression to the variable slope sigmoidal dose–response formula given below:

$$y = \frac{100}{1 + 10^{logIC50-x)H}} \times 100$$

where H is the Hill coefficient or slope factor (Singh & Rosenthal, 2001).

2.5.2.3. Assessment of the changes in biochemical markers of mice blood

Upon completion of the curative test, mice were sedated with the KX cocktail, and their blood was collected in EDTA tubes by sectioning the carotid artery. The collected blood was centrifuged at 3000 rpm at 4 °C for 5 minutes, and the collected supernatant was stored at −20 °C for further analysis of biochemical markers. The LABKIT kit was used to quantify alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, creatinine, and total bilirubin levels.

2.6. Analysis of the histopathological changes

Cross-sectional samples (5 µm) of the liver, spleen, and kidney were dehydrated using ascending grades of alcohol, and 5-micron thick slides were stained with hematoxylin-eosin (HE) (Leica Biosystems) dye as recommended by the manufacturer and examined under a light microscope (at x40 magnification) and photographed using a microscope camera (Axioshop, Germany).

2.7. In silico prediction of ADMET properties of two antiplasmodial phytoconstituents of TiH_2O and putative targets

Our previous studies unveiled ellagic acid and leucodelphidin as the main pharmacophores incriminated in the activity of TiH_2O extract (Tali et al., 2022). Thus, ADMET and drug-likeness properties were predicted using the freely available Swiss ADME (Daina et al., 2017) and pkCSM predictor (Pires et al., 2015) tools. Chemical codes of ellagic acid and leucodelphidin were extracted from the chemical structure and used as input to the Swiss ADME (http://www.swissadme.ch/index.php) and pkCSM predictor (http://biosig.unimelb.edu.au/pkcsm/), to predict pharmacokinetic properties. In addition, plausible molecular targets were retrieved using Swiss target prediction tools (http://www.swisstargetprediction.ch/).

2.8. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Means were compared by analysis of variance (ANOVA), followed by Tukey's post-test using GraphPad Prism version 8.0 and values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. T. ivorensis aqueous extract (TiH_2O) is safe in mice

Oral dose of TiH_2O at 2000 mg/kg b.w. did not cause mortality or major behavioral changes among the experimental groups of animals, indicating its safety as per the criteria of the OECD (OECD, 2002). Additionally, no significant changes in the weight of the TiH_2O-treated mice were recorded (Figure 1).

3.2. TiH_2O displays antimalarial efficacy

3.2.1. TiH_2O halted disease installation in mice

The treatment with TiH_2O extract displayed significant chemo-suppressive activity within 4 days of post parasite inoculation. Like CQ, TiH_2O suppressed *P. berghei* growth by 100% relative to untreated mice (Table 1 and Figure 2A). However, at day 8 of post parasite inoculation, there was a parasitemia shift between the

TiH$_2$O (87.91%) and the CQ (100% suppression) treatments (Table 1 and Figure 2B).

On another hand, treatment of mice with TiH$_2$O resulted in a survival rate close to the chloroquine level up to day 8. Beyond day 9, all infected mice that received the vehicle died, whereas 5 out of the 6 mice (83.30%) were treated with TiH$_2$O and all CQ-treated mice survived until day 15 of the experiment (Figure 2C).
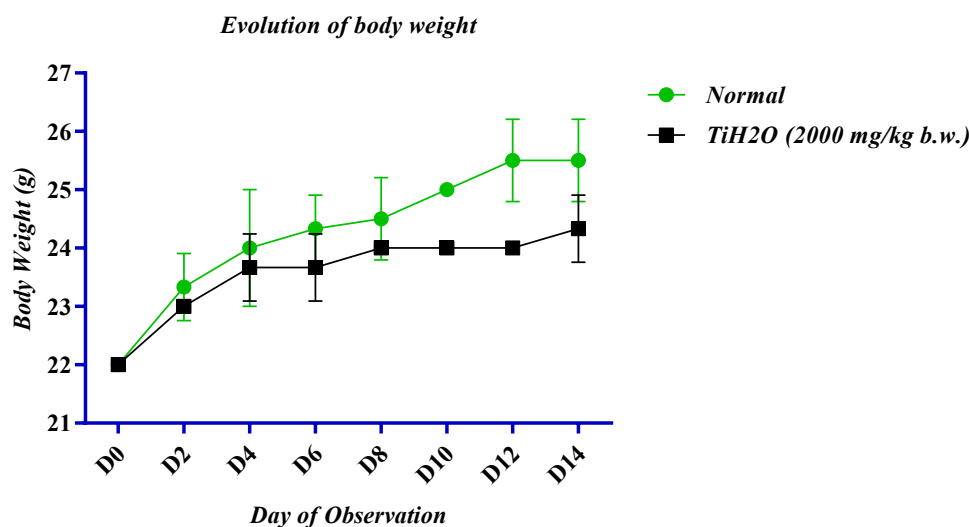
Evolution of body weight

Figure 1. The body weight variation of mice under TiH$_2$O treatment.
Each point represents the mean ± SD; n = 3. TiH$_2$O: Aqueous extract of *T. ivorensis* stem bark. Administered dose: 2000 mg/kg b.w.

Table 1. Percentage of *P. berghei* suppression by TiH$_2$O extract

	Percent parasite suppression (%)	
	Day 4	Day 8
Chloroquine	100 ± 0.00	100 ± 0.00
TiH$_2$O	100 ± 0.00	87.91 ± 0.51***

Percentages of parasite suppression according to the treatment, calculated by comparison to TiH$_2$O-treated mice. ***$p < 0.0001$. TiH$_2$O: Aqueous extract of *T. ivorensis*.
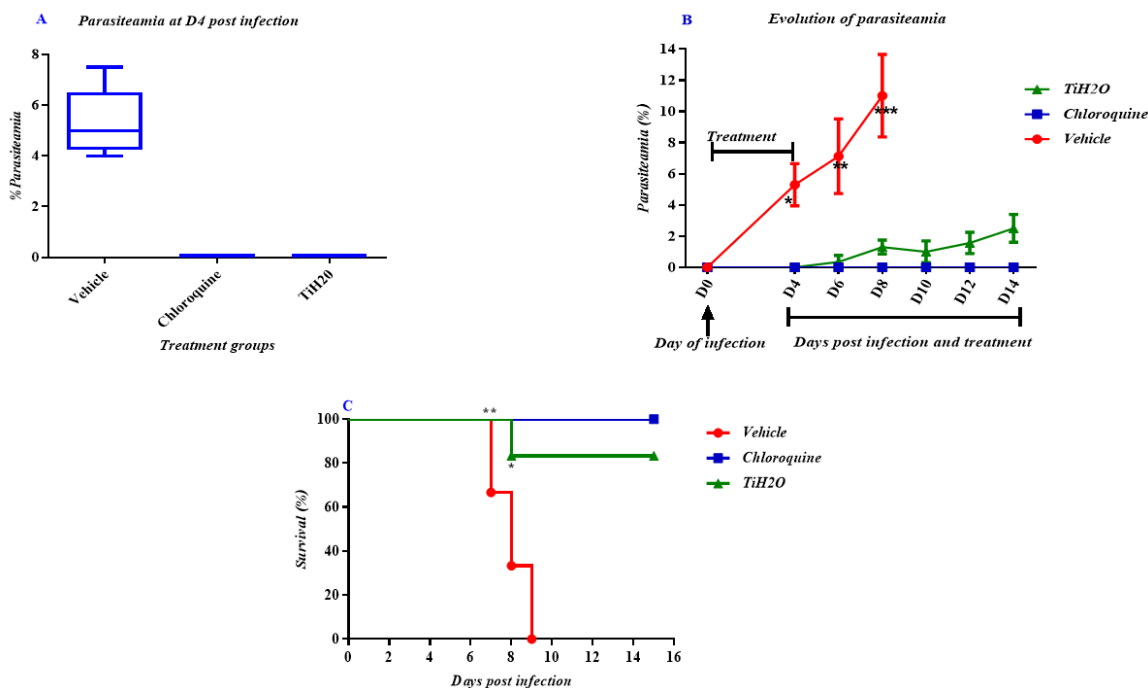

Figure 2. Antimalarial efficacy of TiH$_2$O in the *P. berghei* model
BALB/c mice were infected intraperitoneally with *P. berghei* NK65 (106 parasites/mice) and treated 2 h later with chloroquine (10 mg/kg), TiH$_2$O (100 mg/kg), or water (25 ml/kg) from day 0 to day 3. Parasite densities (parasitemia) were measured every 48 h from D4 to D14. (A) Effects of chloroquine; TiH$_2$O on the parasitemia of infected mice on day 4. (B) Evolution of parasitemia beyond day 4 post-infection (D4 to D14). Parasitemia was compared according to the treatment received (*$p < 0.05$; **$p < 0.005$ or ***$p < 0.001$). TiH$_2$O and CQ treatment were compared to vehicles. (C) Percentage of survival following infection in mice. Survival was measured daily and compared according to treatments received at D9 (**$p < 0.005$). TiH$_2$O and CQ treatment were compared to vehicle (**$p < 0.05$). TiH$_2$O: Aqueous extract of *T. ivorensis* stem bark

3.2.2. TiH₂O extract cures malaria symptoms in a murine model

The TiH$_2$O extract cured established malaria infection and restored key biochemical parameters and mouse histology. Compared to the negative control animals, TiH$_2$O administration at 200 and 400 mg/kg b.w. decreased parasitemia in a dose-dependent manner in *P. berghei*-infected mice from day 4 to day 8 (Figure 3). Compared to

CQ at 10 mg/kg b.w., TiH$_2$O at doses of 100, 200, and 400 mg/kg b.w. inhibited parasite growth by only 88.8%, 97.4%, and 98.05%, respectively (corresponding to percent parasitemia of 11.2, 2.6, and 1.95%) within the same timeframe. Overall, the median efficacy dose (ED$_{50}$) of TiH$_2$O was 96.80 mg/kg b.w.
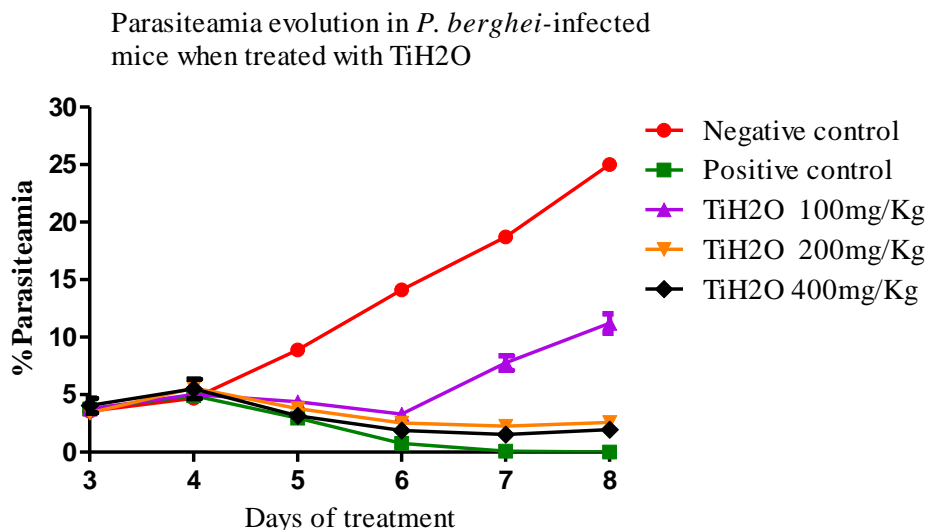

Figure 3. The curative effect of TiH$_2$O on *P. berghei*-infected mice
(A) The parasitemia levels were monitored from day 3 through day 8. TiH$_2$O: Aqueous extract of T. ivorensis stem bark

In summary, TiH$_2$O exhibited a comparable or superior suppressive and curative antimalarial effect at 200 and 400 mg/kg relative to CQ tested at 10 mg/kg. Additionally, in the daily post-drug treatment monitoring of animals, TiH$_2$O exhibited the ability to prolong mice survival, primarily attributable to its effectiveness in treating malaria infection by targeting and eliminating the malarial parasites, contributing to the prolonged survival of the mice. Additionally, the balance of the inflammatory response might contribute to the overall well-being of the animals by potentially impacting their survival. Also, if TiH$_2$O modulates the immune response to reduce excessive inflammation and promote a balanced immune reaction, it could positively influence the overall health of the mice. Indeed, ellagic acid and analogs are widely distributed in *Terminalia* spp., and their potential as anti-inflammatory and immunomodulatory agents is well documented (Abiodun et al., 2016; BenSaad et al., 2017; de Araujo et al., 2019; Deepika & Maurya, 2022). Besides, the observed recrudescence of parasites during daily observation of thin blood smears and post-drug exposure might be linked to latent *P. berghei* merozoites escaping the extract's effect by "hiding" in macrophages and neutrophils (Landau et al., 1999). In addition to our previous report (Tali et al., 2022), many other reports have highlighted the antimalarial efficacy of *Terminalia* species, all containing ellagic acid and derivatives among the main constituents, including Tali et al. (2022) who demonstrated that *T. mantaly* significantly cured murine malaria with ED$_{50}$ of 69.50 mg/kg and LD$_{50}$ above 2000 mg/kg. Similarly, Biruk et al. (2020) reported that an 80/20 methanol/water extract from *T. brownii* demonstrated good safety (LD$_{50}$ > 2000 mg/kg) and significantly suppressed *P. berghei* ANKA proliferation (Biruk et al., 2020). Also, *T. albida* methanol extract was reported to drastically suppress *P. berghei* ANKA parasitemia by 100% (Camara et al., 2019). Moreover, these findings were corroborated by the previously reported activities of the ubiquitous ellagic acid (Njomnang Soh et al., 2012). Of note, there is

no study in the literature that specifically describes the activity of leucodelphidin.

3.2.3. Treatment of mice with TiH₂O normalizes the biochemical parameters

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) tests were conducted to depict liver dysfunctions and toxicological damages following plasmodial infection, and to ascertain treatment efficacy. The results showed a significant increase in ALT and AST activities in the negative control group of mice (infected and untreated), compared to the normal control (uninfected and receiving only vehicle) (Figure 4A and B). Oral administration of TiH$_2$O at 100, 200 and 400 mg/kg b.w. significantly decreased the levels of ALT and AST (***p < 0.001) in mice. Indeed, ALT and AST are enzymes found primarily in the liver, and their levels in the blood are often used as markers of liver function. Changes in these enzyme levels can be indicative of liver damage or dysfunction. In the context of *Plasmodium* infection (malaria) in mice, decreased levels of ALT and AST following drug administration could have therapeutic significance including 1) indication of therapeutic effect in terms of resolving or preventing liver damage associated with the infection; 2) an improvement in liver function, reflecting the drug's efficacy in addressing the underlying liver pathology associated with malaria; 3) a reduction in the parasite load in the liver; and 4) a broader improvement in the health status of the infected mice, indicating a positive response to the antimalarial treatment and corroborating the improved survival rate. Creatinine level significantly increased (**p < 0.05) (Figure 4C) in the negative control group compared to the normal control group. However, administration of CQ at 10 mg/kg/b.w. and TiH$_2$O at 100, 200 and 400 mg/kg b.w. elicited a consistent decrease (**p < 0.05) in blood creatinine thereby depleting the deleterious creatinine elevation elicited by parasite growth. This decrease

further suggests that TiH$_2$O may be effective in mitigating the damage caused by malaria infection, particularly in the liver and kidneys. Similarly, the replication of *P. berghei* in infected mice correlated with a significant increase in bilirubin level compared to the normal group **(Figure 4D)**. This effect was significantly normalized (**$p < 0.001$) by the effect of CQ at 10 mg/kg b.w. and

TiH$_2$O at 200 and 400 mg/kg b.w. **(Figure 4D)**. It should be noted that elevated bilirubin levels can be indicative of hemolysis and liver dysfunction. Therefore, a decrease in bilirubin levels following TiH$_2$O administration suggests potential therapeutic benefits.
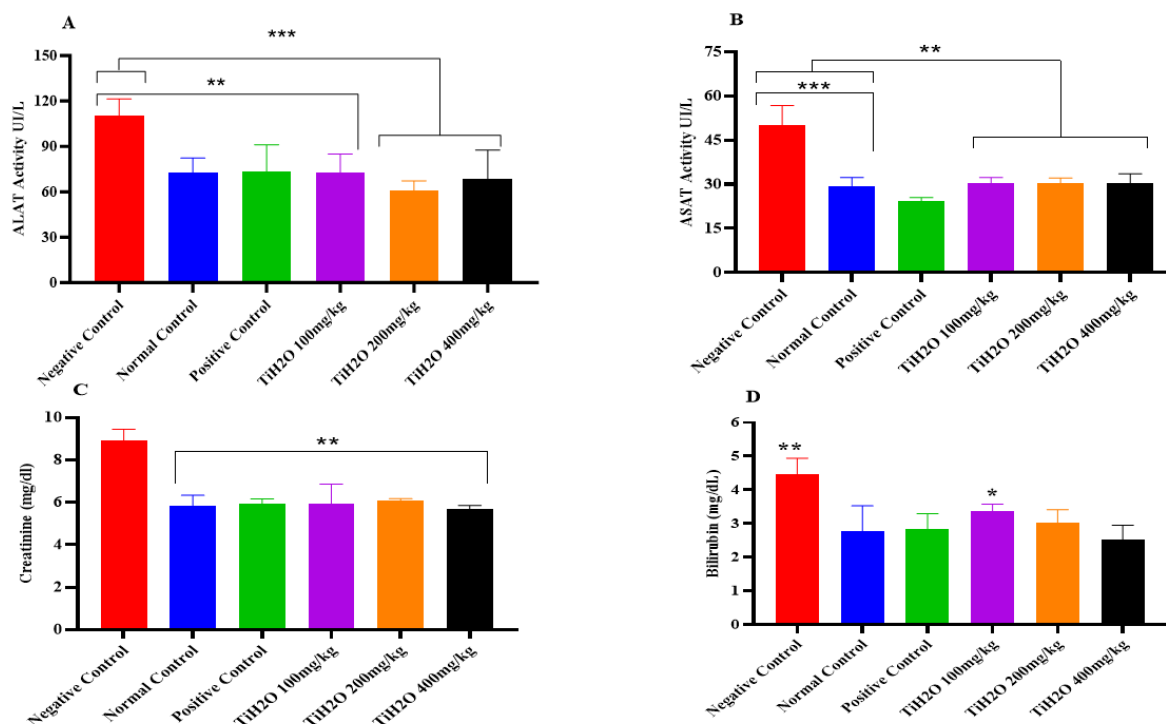
Figure 4. Effect of TiH$_2$O extract on ALT, AST, creatinine activity and bilirubin level

Each bar represents the mean ± SD, $n = 6$, A: Activity of ALT in *P. berghei*-infected mice and normal control groups, **$p < 0.05$; ***$p < 0.001$. B: Activity of AST in *P. berghei*-infected mice and normal control groups, **$p < 0.05$; ***$p < 0.001$. C: Activity of creatinine in *P. berghei*-infected mice and normal control groups, **$p < 0.05$. D: Level of bilirubin in *P. berghei*-infected mice and normal control groups, *$p < 0.05$; **$p < 0.001$

3.2.4. Histopathological profile of TiH$_2$O-treated versus untreated mice

The comparative effects of TiH$_2$O extract, CQ (positive control), and distilled water (negative control) on the microarchitecture of the examined organs indicated that parasite infection resulted in hepatic leukocyte infiltration, glomerulosclerosis in the kidneys, and disruption of the white and red pulp of the spleen in the infected, but untreated mice compared to the uninfected and untreated normal control **(Figure 5)**. All these injuries may be caused by malarial anemia, cytoadherence phenomena, deposits of cell membrane debris, and malarial pigment. These factors lead to severe hypoxia, organ damage, increased lactemia, and shock (da Silva Junior et al., 2017). As a result of treatment with TiH$_2$O extract at doses of 200 and 400 mg/kg b.w. and CQ at 10 mg/kg, significant restoration of histological architecture of the liver, kidney, and spleen was elicited after 5 days of treatment.

3.3. Pharmacokinetics and drug-likeness profiles of leucodelphidin and ellagic acid

The data generated from the ADMET and drug-likeness prediction of leucodelphidin and ellagic acid **(Figure 6)** showed that both compounds satisfy the criteria of Lipinski's rule of five, indicating their drug-likeness. On another hand, leucodelphidin displayed poor absorption (51.01%) compared to ellagic acid (86.68%) **(Table 2)**. Besides, the prediction suggested that ellagic acid and leucodelphidin cannot cross the blood-brain barrier (BBB) and do

not inhibit hERG activity. Also, ellagic acid was predicted to inhibit CYP1A2 contrarily to leucodelphidin. The radar plot **(Figure 7)** indicates limited oral bioavailability for both compounds, probably due to highly unsaturated and polar features. In addition, the estimated plausible molecular targets for both compounds **(Figure 8)** indicated a preferential inhibition of kinases (60%) for ellagic acid followed by lyases (20%), while leucodelphidin was predicted to inhibit lyases (40%), followed by kinases (33.33%). Other less significant targets included the family of AG protein-coupled receptors (6.7%) and cytosolic proteins (6.7%) for ellagic acid, and proteases (13.3%), oxidoreductases (6.7%), and primary active transporters (6.7%) for leucodelphidin.

The predicted properties indicated that ellagic acid and leucodelphidin meet promising pharmacokinetics and drug-likeness properties, suggesting that both compounds possess characteristics that make them promising candidates as pharmaceutical compounds. Thus, further optimization might improve their ADME properties crucial for effectiveness in the body; their bioavailability to imply that enough of the compounds reaches the bloodstream and target tissues, thereby increasing the likelihood of a therapeutic effect; their metabolism such that by-products do not elicit toxicity. Besides, target prediction has unveiled plasmodial kinases and lyases as the main possible targets of ellagic acid and leucodelphidin. Notably, kinases are a class of molecular drug targets extensively investigated in multiple disease areas, including malaria as exemplified by the *P. falciparum* phosphatidylinositol 4-kinase type III beta (PfPI4KIIIβ) targeted by an inhibitor currently in

clinical development for malaria treatment (Paquet et al., 2017; Sinxadi et al., 2020). Kinases are critically involved in the development of *P. berghei* in mosquitoes (Tewari et al., 2010), and in the asexual blood stage in *P. falciparum* (Solyakov et al., 2011).

Additional studies are therefore warranted to bring insights into the actual targets of both compounds.
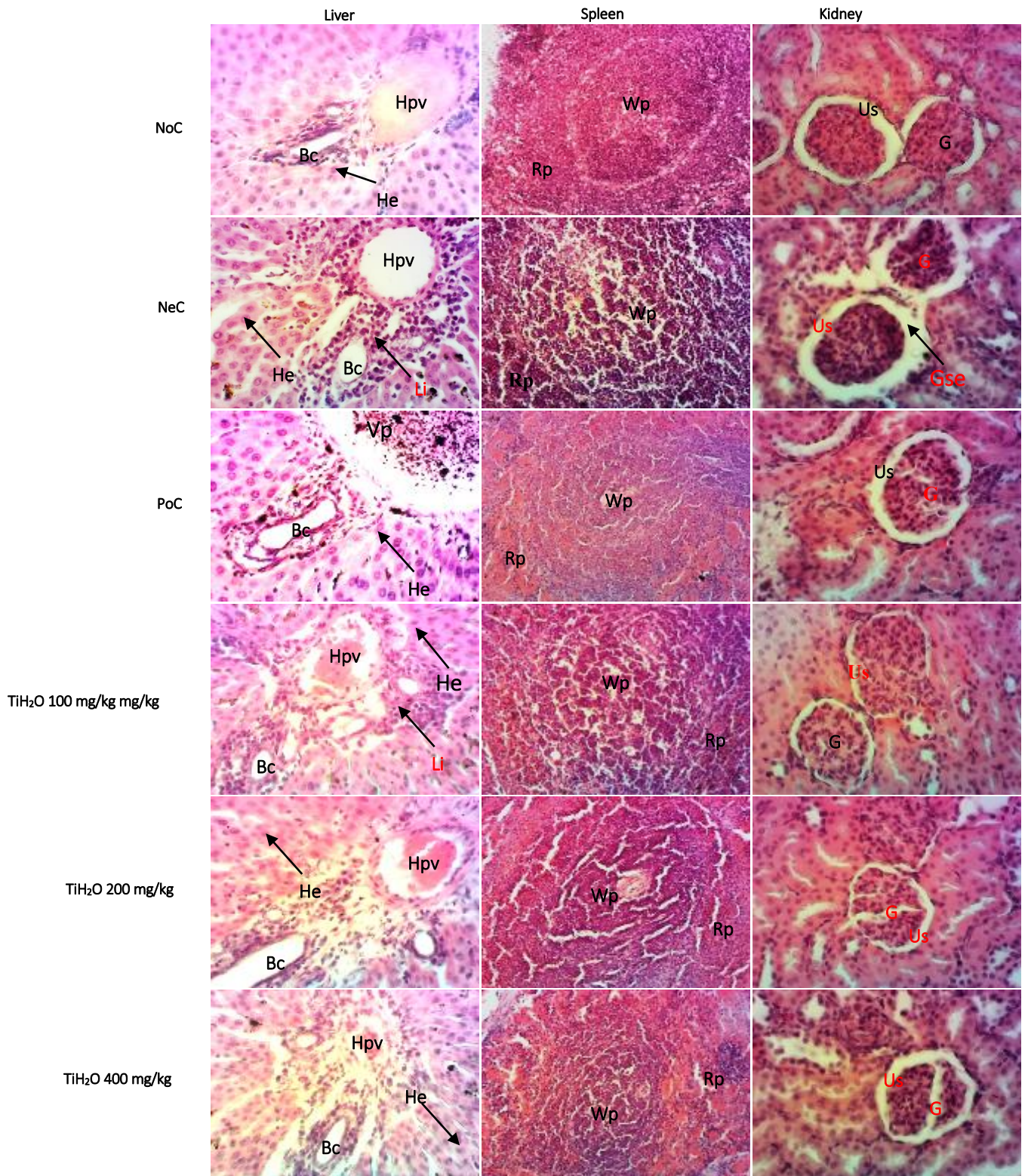

Figure 5. Micrography of liver, spleen, and kidney sections (HE x 400) of *P. berghei*-infected mice treated with TiH₂O (100, 200, 400 mg/kg), and CQ (10 mg/kg)

Hpv: Hepatic portal vein, He: Hepatocytes, Bc: Biliary canaliculus, Li: Leukocyte inflammation, Wp: White pulp, Rp: Red pulp, G: Glomerule, Us: Urinary space, Gse: Glomerulosclerosis, NoC: Normal control, NeC: Negative control, PoC: Positive control
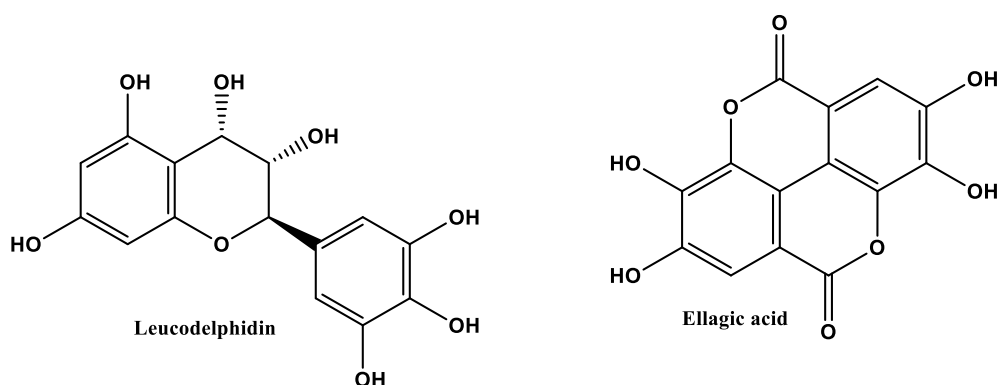
Figure 6. Pharmacophores reported as the main antiplasmodial ingredients of TiH$_2$O (Tali et al., 2022)

Table 2. Predicted pharmacokinetics and drug-likeness of leucodelphidin and ellagic acid

Property	Parameters	Predicted value		Unit/Classifications
		Leucodelphidin	Ellagic acid	
Drug-Likeness (MW < 500; HBD ≤ 5; HBA ≤ 10; logP ≤ 5)		Yes	Yes	
Absorption	Caco2 permeability	-2.916	-3.181	Numeric (log mol/l)
	Intestinal absorption (human)	-0.059	0.335	Numeric (log Papp in 10^{-6} cm/s)
	Skin Permeability	51.018	86.684	Numeric (% Absorbed)
	P-glycoprotein substrate	-2.735	-2.735	Numeric (log Kp)
	P-glycoprotein I inhibitor	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	No	Categorical (Yes/No)
Distribution	VDss (human)	1.721	0.375	Numeric (log l/kg)
	Fraction unbound (human)	0.486	0.083	Numeric (Fu)
	BBB permeability	-1.185	-1.272	Numeric (log BB)
	CNS permeability	-3.601	-3.533	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	Categorical (Yes/No)
	CYP3A4 substrate	No	No	Categorical (Yes/No)
	CYP1A2 inhibitor	No	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	No	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	No	Categorical (Yes/No)
Excretion	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
Toxicity	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
	Total clearance	0.216	0.537	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)

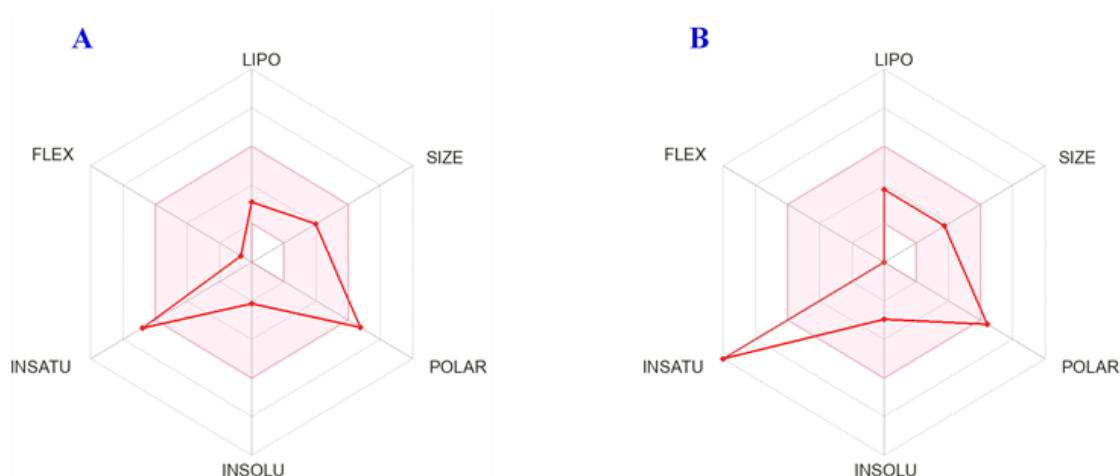hERG: Human ether ago-ago gene, CYP: Cytochrome P450

Figure 7. Bioavailability radar plots of (A) leucodelphidin and (B) ellagic acid

LIPO: Lipophilicity (between – 0.7 and + 5.0), SIZE: Molecular weight (between 150 and 500 g/mol), POLAR: Polarity (between 20 and 130 Å2), INSOLU: Solubility (≤ 6),
INSATU: Saturation (fraction of carbons in the sp3 hybridization ≥ 0.25), FLEX: Flexibility (≤ 9 rotatable bonds)
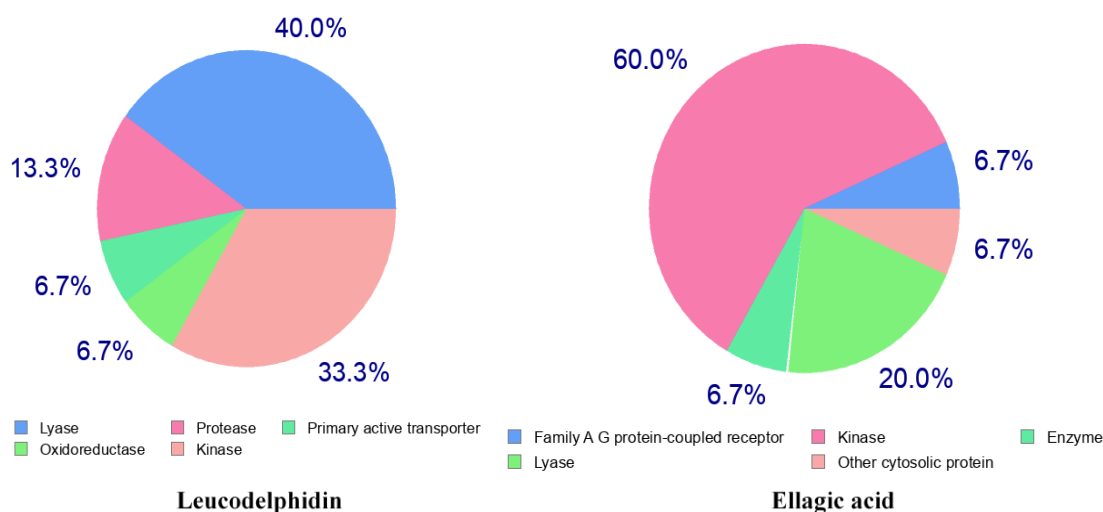
Figure 8. Pie chart of the top 50% of putative target prediction for ellagic acid and leucodelphidin

4. Conclusions

By amalgamating the previously reported in vitro antiplasmodial potency and the in vivo data reported herein, the outcome compellingly supports the conclusion that *T. ivorensis* could serve as a reliable source of new promising pharmacophores for antimalarial drug discovery. However, limitations include a lack of mechanistic insights to understand the actual mode of action of *T. ivorensis* metabolites against malaria parasites. Thus, in-depth exploration is warranted to comprehensively profile the metabolome and other biological parameters of this extract or their chemical isolates. In addition, this analysis adds to the continuous validation of *T. ivorensis* to treat malaria.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

Ethical approval for this study was obtained from the Joint Institutional Review Board for Animal & Human Bioethics (JIRB), University of Yaounde 1, Cameroon (No: BTC-JIRBI2023-091).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Eugenie Aimée Madiesse Kemgne: Investigation, Methodology, Draft review
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Supplementary File

None.

Publisher's Note

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