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Preclinical assessment of the acute hematological safety of an antimicrobial ethanolic phytoextract from *Combretum racemosum* in Wistar rats

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Abstract

Many ethanolic phytoextracts exhibit promising antimicrobial performance but may also display toxic effects, particularly on blood erythrocytes, potentially leading to acute hemolytic anemia. Therefore, it is essential to verify their safety. This study aimed to evaluate the acute erythrocyte safety of an ethanolic phytoextract (Ethe) with antimicrobial potential from *Combretum racemosum* in female Wistar rats, by assessing the potential risk of acute hemolytic anemia. Eighteen female rats (n = 18) were divided into six groups of three animals each (n = 3) and orally administered single doses of Eethe at 5, 50, 300, 2000, and 5000 mg/kg of body weight, while the negative control group (n = 3) received distilled water. Red blood cell (RBC) count, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured 24 hours after Ethe administration. No significant clinical abnormalities were observed at 5% threshold ($p > 0.05$), regardless of the single dose administered. The values of RBC, Hb, Hct, MCV, MCH, and MCHC remained stable within their physiological reference ranges. No significant variations indicative of hemolytic anemia were detected at 5% threshold ($p > 0.05$). The ethanolic phytoextract (Ethe) from *Combretum racemosum* leaves tested in this study exhibited good single-dose safety on the erythrocytes of Wistar rats, even at high doses (up to 5000 mg/kg of body weight). However, subchronic and chronic studies are required to confirm this observation and support the development of Ethe as a natural antimicrobial candidate.

Keywords: Phytoextract; *Combretum racemosum*; Antimicrobial activity; Hematological safety; Preclinical

1. Introduction

The human species, along with its main activities, particularly modern agriculture (both plant and animal), is increasingly confronted with persistent microbial infections that compromise human, animal, and plant health. These infections lead to reduced productivity, increased food insecurity, and pose a threat to consumer health [1].

Infectious diseases remain one of the major causes of morbidity and mortality worldwide. They also result in decreased agricultural production, higher veterinary costs, and substantial economic losses for farmers. Such disruptions affect supply chains, influence commodity prices, and generate broader economic repercussions, especially in agricultural sector [2].

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The fight !against these infections still largely relies on the use of synthetic drugs (antibiotics, antifungals, and pesticides). However their intensive use promotes the emergence of resistant microbial strains and raises significant ecotoxicological concerns [3–4].

In this context, medicinal plant extracts appear as a sustainable alternative. Their efficacy relies on their richness in natural secondary metabolites with antibacterial and antifungal activities, particularly when obtained through ethanol extraction [5]. Nevertheless, before any therapeutic application, it is essential to assess the safety of these extracts on living organisms, especially on the blood system. Red blood cells, due to their central role in oxygen and carbon dioxide transport via hemoglobin, serve as sensitive biomarkers of toxicity [6–7].

Among plants of interest, *Combretum racemosum* (family Combretaceae) is recognized for its diverse biological activities. It has demonstrated antidermatophytic and anthelmintic efficacy [8–9], as well as activity against trypanosomal and antiplasmodial infections [10]. Its anti-inflammatory and antioxidant properties are also well documented [11–12], along with its therapeutic potential for urinary and gastrointestinal infections [13].

However, the occurrence of erythrocyte disturbances associated with signs of hemolytic anemia following administration of a drug substance may constitute a major risk limiting its therapeutic use.

Therefore, the present study aimed to evaluate the acute preclinical safety of an ethanolic leaf phytoextract of *Combretum racemosum*, known for its antimicrobial activity, on red blood cells, in order to assess its potential hemolytic toxicity and biological compatibility.

2. Material and methods

2.1. Tested phytoextract

The tested phytoextract consisted of an ethanolic macerate (Ethe). It was obtained from the leaves of *Combretum racemosum* (Figure 1), which were authenticated at the National Floristic Center of Félix Houphouët-Boigny University, Abidjan-Cocody, by comparison with specimen No. 16949, deposited on July 17, 1985.

Figure 1 Leaves of *Combretum racemosum*

2.1.1. Preparation of the ethanolic phytoextract

After authentication and preliminary processing, the leaves of *Combretum racemosum* were sorted, washed, and air-dried for one week. The dried leaves were then ground into a fine powder, which was stored in sterile containers at 20 °C for the preparation of the ethanolic phytoextract (Ethe).

Ethe was obtained by macerating 100 g of the leaf powder in a water/ethanol mixture (300 mL/700 mL), homogenized at 37 °C, then filtered and concentrated at 50 °C for one week [13].

2.1.2. Phytochemical test of the ethanolic phytoextract

Ten colorimetric assays performed on the ethanolic phytoextract (Ethe) revealed, through reduction reactions in basic medium, the presence of biologically active substances, as confirmed by the appearance of specific coloration reactions [14].

2.1.3. Sterility test of the ethanolic phytoextract

The microbiological quality control of the ethanolic phytoextract (Ethe) was carried out in accordance with established standards [15]. This test aimed to determine whether the Ethe was contaminated by any microorganism. The qualitative microbiological assessment covered all types of microorganisms.

2.2. Hematological components studied

The blood components analyzed concerned erythrocytes obtained from young healthy female Wistar rats (n = 18), aged eight weeks and with a mean body weight of 100 g. The species used, Rattus norvegicus, belongs to the Muridae family (Figure 2).

Figure 2 *Rattus norvegicus* species

2.3. Preclinical evaluation of the acute safety of the ethanolic phytoextract on the erythrocyte parameters of treated female rats

2.3.1. Oral administration (gavage) of tats with the ethanolic phytoextract

The preclinical study of the acute safety of the ethanolic phytoextract (Ethe) on the erythrocytes of female rats was conducted according to the OECD Guideline 423, as validated and described by [16].

Eighteen female rats, after a 12-hour fasting period (with free access to water), were divided into six groups of three animals each (n = 3) : a negative control group that received 1 mL/100 g of distilled water; three test groups (n = 9) that received the trial doses (5, 50, and 300 mg/kg of body weight); a “limit-tolerated” group at 2000 mg/kg of bw; and a “limit” group at 5000 mg/kg of bw. The Ethe was administered once orally by gavage. After gavage, the animals were fasted for one additional hour before being re-fed. The blood erythrocyte parameters were then measured following this single-dose administration.

2.3.2. Observation of acute physical and behavioral clinical signs in treated female rats

After administration of the ethanolic phytoextract (Ethe), the rats were observed immediately during the following 30 minutes, at 4 hours, 24 hours, and then daily for 14 days [17].

Physical clinical signs were recorded based on the condition of the skin, mucous membranes, urine, feces, and hair coat, followed by behavioral signs relating to breathing, salivation, lethargy, convulsion, aggressiveness, drowsiness, feeding behavior, locomotion, stretching, and mortality. The number of deaths was also monitored in all animals.

2.3.3. Blood sampling from rats treated with the phytoextract

At the end of the treatment, all rats were sacrificed. Blood samples were collected in EDTA tubes for complete blood count (CBC) analysis, which allowed the determination of total red blood cell concentration, hemoglobin level, and erythrocyte indices (MCV, Hct, MCHC, MCH), according to the method recommended for hematological analysis in rats [18].

2.3.4. Determination of total red blood cells and hemoglobin levels

Total red blood cells (RBCs) were converted into isovolumetric spheres and counted using the **Technicon H1** analyzer. Their quantification was supplemented by electronic pulse counts with the **Coulter Counter S-PLUS IV.** Hemoglobin (Hb) levels were determined using the modified cyanmethemoglobin method, in accordance with current standards [19].

2.3.5. Measurement of erythrocyte indices

The erythrocyte constants (MCV, Hct, MCH, and MCHC) were automatically measured using a calibrated hematology analyzer, ensuring accuracy and reproducibility [20].

The calculation of their values was performed using the following standard formulas :

$$Hct = \frac{MCV}{RBC} \times 100 \; ; \; MCV = \frac{Hct}{RBC} \times 10 \; ; \; MCH = \frac{Hb}{RBC} \times 10 \; ; \; MCHC = \frac{Hb}{RBC} \times 100$$

MCV : Mean Corpuscular Volume (fL)

RBC : Number of red blood cells per unit of blood (10^{12}/L of blood).

MCH : Mean corpuscular hemoglobin (pg)

MCHC : Mean corpuscular hemoglobin concentration (g/dL)

Hb: Hemoglobin concentration (g/dL ou g/L).

2.4. Statistical analysis

Phytochemical and acute safety data of Ethe were analyzed using **GraphPad Prism** and **R**, and are presented as mean ± SE. Statistical significance was assessed by **ANOVA** followed by the **Student's t-test**, with a significance threshold set at $p < 0.05$.

3. Results

3.1. Groups of bioactive phytomolecules identified in the ethanolic phytoextract

The analyzed ethanolic phytoextract (Ethe) contains three (03) groups of biologically active phytomolecules (Table 1). The first group, represented by flavonoids, total polyphenols, and free quinones, is absent in Ethe. The second group, less abundant in Ethe, consists of tannins and saponins. The third group, comprising alkaloids, steroids, and terpenoids, is present in high amounts in Ethe.

In summary, the ethanolic phytoextract contains **alkaloids, saponins, steroids, terpenoids, and tannins** (catechic and gallic).

Table 1 Profile of bioactive phytomolecules identified in the ethanolic phytoextract (Ethe) from Combretum racemosum leaves

Polyextract	Alkaloids	Flavonoids	Total polyphenols	Free quinones	Saponins	Stéroïdes	Terpénoïdes	Tanins	
								Cat	Gal
Ethe	++	-	-	-	+	++	++	+	+

Ethe : Crude hydroethanolic macerated phytoextract ; B : Bouchardat ; Cat : Catechic tannins ; Gal : Gallic tannins ; - : Absence of Bioactive phytomolecules ; + : Presence of bioactive phytomolecules

3.1.1. Physical and behavioral manifestations observed in treated female rats

Within 24 hours following administration of the ethanolic phytoextract (Ethe), no abnormal physical or behavioral manifestations were observed in the treated female rats (Table 2). The animals' physical appearance including the condition of the skin, fur, mucous membranes, eyes, ears, and mouth as well as their general behavior, such as respiration, locomotion, salivation, lethargy, convulsions, aggressiveness, and responsiveness to auditory and tactile stimuli, remained comparable to those of the control group.

At the highest tested dose (5000 mg/kg of body weight), Ethe did not induce any acute toxic effects related to physical appearance or behavior that could indicate hemolytic anemia in the treated female rats.

In summary, administration of Ethe produced no signs of acute physical or behavioral toxicity suggestive of acute hemolytic anemia.

Table 2 Acute clinical signs related to physical appearance and behavior observed in treated female rats

Observed parameters	**Control group**				**Groups of treated female rats**			
Physical appearance	**30 min**	**4 h**	**24 h**	**14 days**	**30 min**	**4 h**	**24 h**	**14 days**
Skin and fur		**-**	**-**	**-**	**-**	**-**	**-**	**-**
Mucous membranes	-	-	-	-	-	-	-	-
Mouth, ears, eyes	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
General behavior								
Arythmia		-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-
Léthargy	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-
Agressiveness	-	-	-	-	-	-	-	-
Sleep	-	-	-	-	-	-	-	-
Locomotor activity	+	+	+	+	+	+	+	+
Feeding	+	+	+	+	+	+	+	+
Stretching	-	-	-	-	-	-	-	
Agitation	-	-	-	-	-	-	-	

General appearance cases : - : Normal, + : Abnormal; Case of général behavior : - : Absence; + : Presence.

3.1.2. Acute erythrocytic effect of the phytoextract in female rats

A single oral administration of the ethanolic phytoextract (Ethe) of *C. racemosum*, rich in saponins, steroids, terpenoids, and tannins, at doses ranging from 5 to 5000 mg/kg body weight, did not induce any significant acute harmful effect on circulating RBCs, hemoglobin (Hb), or hematocrit (Hct) at the 5% significance threshold ($p > 0.05$) compared with the control group (Table 3). The counts of RBCs, Hb, and Hct remained within normal ranges and showed no statistically significant difference relative to the control ($p > 0.05$).

RBC counts remained stable from Day 1 (D1) to Day 14 (D14) of the study, varying non-significantly from 7.49 ± 0.12 to 7.78 $\times 10^{12}$ ± 0.32/L in treated rats, compared with 7.80 $\times 10^{12}$ ± 0.21/L in controls (Table 3). Similarly, Hb concentration fluctuated between 12.99 ± 0.31 and 13.90 ± 0.47 g/dL in treated rats, compared with 13.30 ± 0.58 g/dL in controls. Hematocrit values ranged from 40.90 ± 3.37% to 45.08 ± 3.42% in treated rats, versus 41.05 ± 2.89% in the control group.

In summary, Ethe did not cause premature destruction of circulating red blood cells in the studied rats up to the limit dose of 5000 mg/kg body weight.

Table 3 Acute erythrocyte safety profile of Ethe in treated female rats

	Eethe dose (mg/kg of bw)	n	RBC (10^{12}/L)	Hb (g/dL)	Hct (%)
Control group	0	3	7.80 ± 0.21[a]	13,30 ± 0,58[b]	41.05±2,89[c]
Ethe-treated groups	5	3	7.70 ± 0.53[a]	12.99 ± 0.31[b]	42.85±3.27[c]
	50	3	7.49±0.12[a]	13.0±0.25[b]	40.90±3.37[c]
	300	3	7.65±0.22[a]	13.60±0.33[b]	43.03±3.37[c]
	2000	3	7.60±1.21[a]	13.87±0.45[b]	43.03±3.40[c]
	5000	3	7.78±0.32[a]	13.90±0.47[b]	45.08±3.42[c]

RBC : red blood cells; Hb: hemoglobin. **Hct**: hematocrit; Ethe: macerated crude hydro-ethanolic phytoextract. In the same column, RBC, Hb, and Ht marked with the same letter are not significantly different at the 5% threshold ($p > 0.05$).

3.1.3. Acute impact of phytoextract disruption on erythrocyte indices in treated rats

On day 14 (D14) after a single oral administration of Ethe *C. racemosum*, rich in tannins, saponins, steroids, and terpenoids, at doses of 5 to 5000 mg/kg of bw, erythrocyte constants remained within the usual physiological values even at 5000 mg/kg of bw (Table 4). No significant differences were observed for MCV, MCHC, and MCH at the 5% threshold ($p > 0.05$). The constants remained stable within the physiological reference ranges for Wistar rats. The MCV ranged from 55.93±1.42 to 58.10±2.00 fL in treated rats, with no significant difference from the control (57.92±2.44 fL). The TCMH and CCMH varied between 16.09±0.11 and 17.62±0.36 pg and 30.7±0.46 and 31.7±1.75 g/dL, respectively, in the treated groups, compared to 17.82±0.55 pg and 30.7±0.43 g/dL in the controls.

In summary, Ethe has no acute harmful impact on erythrocyte constants, confirming the absence of any premature destructive effect of Ethe on circulating red blood cells in rats.

Table 4 Acute safety profile of Ethe on erythrocyte parameters in treated female rats

	Eeth dose (mg/kg of bw)	n	MCV (fL)	MCH (pg)	MCHC (g/dl)
Control group	0	3	57.92±2.44[a]	17.82±0.55[b]	30.7±0.43[c]
Eeth-treated groups	5	3	57.80±2.02[a]	17.62±0.36[b]	30.7±0.46[c]
	50	3	55.93±1.42[a]	16.63±0.34[b]	31.7±1.75[c]
	300	3	57.93±2.30[a]	16.13±0.15[b]	30.8±1.74[c]
	2000	3	57.52±2.00[a]	16.13±0.15[b]	30.8±1.00[c]
	5000	3	58.10±2.00[a]	16.09±0.11[b]	30.8±1,00[c]

MCV: mean corpuscular volume. **MCH** : mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration. mg/kg of bw: milligram per kilogram of body weight. Ethe : macerated ethanolic phytoextract. In the same column, the MCV, MCH and MCHC values marked with the same letter are not significantly different at the 5% threshold ($p > 0.05$).

3.1.4. Acute erythrocyte safety via investigation of acute hemolytic anemia in treated female rats

The investigation of acute safety of the ethanolic phytoextract (Ethe) on the blood erythrocytes of the rats studied was carried out by comparing the values of the erythrocyte parameters of the studied rats.

The acute safety of the ethanolic phytoextract (Ethe) on the blood erythrocytes of the rats studied was investigated by comparing the erythrocyte parameter values with the characteristic profile of the main forms of hemolytic anemia. No significant sharp decrease in RBC, Hb, and Hct was caused in rats treated with Ethe at the 5% threshold ($p > 0.05$) compared to the control group, thus indicating the absence of acute hemolytic anemia, i.e., indicating the acute safety of Ethe blood erythrocytes in this study.

In summary, the tested Ethe preserves the integrity, i.e., the safety of erythrocytes in Wistar rats, even at high doses (up to 5000 mg/kg of bw).

Table 5 Acute safety profile of Ethe on erythrocyte parameters in treated female rats

Dose (mg/kg.pc)	n	RBC (10^{12}/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
0 (témoin)	3	7.80±0.21^{a}	13.30±0.58^{b}	41.05±2.89^{c}	57.92±2.44^{d}	17.82±0.55^{e}	30.7±0.43^{f}
5	3	7.70±0.53^{a}	12.99 ± 0.31^{b}	42.85±3.27^{c}	57.80±2.02^{d}	17.62±0.36^{e}	30.7±0.46^{f}
50	3	7.49±0.12^{a}	13.50±0.25^{b}	40.90±3.37^{c}	55.93±1.42^{d}	16.63±0.34^{e}	31.7±1,75^{f}
300	3	7.65±0.22^{a}	13.60±0.33^{b}	43.03±3.37^{c}	57.93±2.30^{d}	16.13±0.15^{e}	30.8±1.74^{f}
2000	3	7.60±1.21^{a}	13.87±0,45^{b}	43.03±3.40^{c}	57.52±2.00^{d}	16.13±0.15^{e}	30.8±1.00^{f}
5000	3	7.78±0.32^{a}	13.90±0.47^{b}	45.08±3.42^{c}	58.10±2.00^{d}	16.09±0.11^{e}	30.8±1.00^{f}

RBC: red blood cells; **Hb:** hemoglobin; **Ht:** hematocrit; **MCH:** mean corpuscular hemoglobin; **MCHC:** mean corpuscular hemoglobin concentration. **mg/kg of b.w.:** milligram per kilogram of body weight**. Ethe:** macerated ethanolic phytoextract. Within the same column, MCV, MCH, and MCHC values followed by the same letter are not significantly different at the 5% level ($p > 0.05$).

4. Discussion

4.1. Groups of bioactive phytomolecules identified in the ethanolic phytoextract

The ethanolic phytoextract (Ethe) contains alkaloids, saponins, steroids, terpenoids, and tannins in varying concentrations. The moderate presence of tannins and saponins in Ethe could be explained by the moderate affinity of ethanol for these molecules [21]. The abundance of alkaloids, steroids, and terpenoids in Eeth is justified by the increased polarity of the solvent mixture resulting from the addition of water, which facilitates the extraction of these more lipophilic compounds [22].

4.2. Physical and behavioral manifestations observed in treated female rats

During the 24 hours following administration of the ethanolic phytoextract (Ethe), no abnormal physical or behavioral manifestations were observed in the treated female rats. This could indicate that Eethe has no clinically toxic effect suggestive of hemolytic anemia and appears to be well tolerated in the short term. This result is consistent with several recent studies on the Combretum genus. For example, the ethanolic extract of *C. racemosum* administered orally to albino rats exhibited an LD_{50} greater than 5500 mg/kg of b.w., without causing mortality or clinical signs of toxicity [23]. This observation suggests that even at the highest dose (5000 mg/kg of b.w.), Ethe does not induce any preliminary behavioral or physical signs of acute hemolytic anemia in rats, thereby demonstrating good short-term tolerance, which aligns with the available data on Combretum species.

4.3. Acute erythrocytic effect of the ethanolic phytoextract in treated female rats

The ethanolic phytoextract (Ethe) does not cause premature destructive effects on circulating erythrocytes in the blood of treated rats, even at the limit dose of 5000 mg/kg of b.w. The stable values of RBC, Hb, and Hct indicate that Ethe does not affect RBC production in the bone marrow, nor does it alter the erythrocyte membrane or morphology, i.e., it does not induce hemolysis. Ethe also does not disturb Hb metabolism or oxidation, meaning it does not interfere with Hb synthesis under the experimental conditions tested. Hb and Hct are sensitive hematological indices of alterations related to hemolysis or bone marrow suppression [24].

The absence of any immediate deleterious effect of Ethe may be explained by the combined membrane antioxidant activity of tannins, the dose-dependent effects of saponins (which remain non-toxic at the tested concentrations), and the additional antioxidant contribution of steroids and terpenoids [25–26]. A similar result was reported by [27], showing that administration of a polyphenolic extract in rats did not significantly alter these parameters, indicating a good hematological tolerance profile. The present study describes the phytochemical profile (alkaloids, saponins, tannins, steroids, terpenoids, etc.) and provides a table of hematological parameters (RBC, Hb, Hct, MCV, MCH, MCHC), showing no statistically significant variations between treated groups and the control group at the time of sampling.

4.4. Acute impact of the ethanolic phytoextract on erythrocytic indices in treated female rats

The ethanolic phytoextract (Ethe) produced no acute harmful alterations in erythrocytic indices, confirming the absence of any premature destructive effect of Ethe on circulating red blood cells in the treated rats. This result indicates the

absence of any detectable acute microcytosis, macrocytosis, or hypochromia in the tested rat population and at the administered doses at the time of blood collection. It therefore suggests a good profile of acute erythrocyte safety for Ethe.

These indices are useful biomarkers for identifying erythrocytic disorders related to oxidative stress or membrane damage. They show that Eeth has neither deleterious nor stimulatory effects on red blood cell mass or hematopoiesis, nor any deficiency-related effect on hematopoiesis or iron metabolism. The extract can thus be considered normocytic—acting normally on red blood cell size and morphology and normochromic neither increasing nor significantly decreasing the hemoglobin concentration within erythrocytes [25].

This result could be attributed to the combined action of the endogenous antioxidant system (SOD, catalase, glutathione peroxidase) and the protective effects of saponins, steroids, terpenoids, and tannins, which together prevent any significant alterations [28]. This finding is consistent with that of [29], who reported that the ethanolic extract of Azadirachta indica reduced erythrocyte membrane lipid peroxidation by 35.6% and increased cell viability by 42% ($p < 0.05$).

4.5. Acute safety of Ethe on blood erythrocytes in treated female rats

The tested ethanolic phytoextract (Ethe) preserved the acute integrity of erythrocytes in the studied Wistar rats, even at high doses (up to 5000 mg/kg of b.w.). This confirms the single-dose safety of Eethe on blood erythrocytes. The result indicates the absence of short-term hemolytic anemia and demonstrates a good capacity of hemoglobin (Hb) to transport O_2 from the lungs to the tissues and, conversely, CO_2 from the tissues back to the lungs. It also confirms the absence of microcytic, macrocytic, or hypochromic anemia.

This observation may be explained by the antioxidant, protective, anti-hemolytic, or stabilizing effects exerted by tannins present in Eeth on animal blood erythrocytes [30]. Indeed, tannins have been shown to protect erythrocytes from damage induced by reactive oxygen species (ROS) generated during oxidative stress, such as peroxynitrite ($ONOO^-$) and hypochlorite (HClO). They enhance the activity of antioxidant enzymes, inhibit lipid peroxidation, restore reduced glutathione and superoxide dismutase levels in obese rats, decrease erythrocyte osmotic fragility, and stabilize erythrocyte membranes through interactions with membrane proteins and a reduction in surface membrane fluidity. These effects improve resistance to osmotic stress and prevent hemoglobin oxidation [31–33].

5. Conclusion

This preclinical study evaluated the acute safety of an ethanolic phytoextract (Ethe) with antimicrobial potential, focusing on the risk of acute hemolytic anemia in female Wistar laboratory rats.

A single oral administration of Ethe at doses ranging from 5 to 5000 mg/kg of b.w. produced no clinical signs of acute hemolytic anemia an essential criterion confirming the single-dose safety of Ethe in this study. This was supported by the absence of any acute hematotoxic effect on RBC, Hb, Hct, MCV, MCH, and MCHC values over a 14-day period. However, the small sample size (n = 3 per group), the use of a single-dose protocol, and the limited experimental duration represent methodological constraints.

Nevertheless, a complementary repeated-dose oral gavage study in Wistar rats, followed by multi-week observations, measurement of hemolytic anemia biomarkers, and histopathological analysis, would be necessary to confirm the hematological safety of Ethe and support its therapeutic development as a potential natural antimicrobial candidate.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest to be reported.

Statement of ethical approval

This research study was conducted on animal, specifically laboratory Wistar rats on the species *Rattus norvegicus*. Its does not contain an appropriate ethical approval statement. Currently, an appropriate ethical approval statement does not exist for research in Ivory Cost. It is currently being developed

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