

Assessment of the Phytochemical Constituents of Methanol Extract of *Ereromastax Polysperma* Leaves and its Effect on the Hematological Indices in Albino Rats

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Abstract

The study investigated the effects of chronic administration of methanol extract of *Ereromastax polysperma* on the hematological parameters of albino rats. The rats were administered daily and orally for 14 days, with water as a control. On day 15, they were anesthetized using chloroform and blood was withdrawn from the heart through cardiac puncture into ethylene diamine tetra acetic acid (EDTA) specimen bottles. The phytochemical screening of crude methanolic extracts revealed the presence of secondary metabolites such as alkaloids, saponin, flavonoids, and cardiac glycosides. The statistical test employed was Analysis of Variance (ANOVA), with a predetermined significance level of $p < 0.05$ and $p < 0.01$. The ANOVA analysis revealed significant differences in red blood cell count (RBC), hemoglobin (HGB) percentages among the control group and the treatment groups of 500mg/kg and 1500mg/kg. The hematological evaluation showed a significant difference in RBC, HGB, and HCT, and a significant decrease in mean corpuscular hemoglobin concentration (MCHC) with increasing doses of the extract. The results suggest that *E. polysperma* has anti-inflammatory, antimalarial, antimicrobial, cytotoxic, antispasmodic, and pharmacological effects, potentially aiding in disease treatment.

Keywords: *Ereromastax polysperma*; Phytochemical; Haematology; Anti-inflammatory; Flavanoids.

INTRODUCTION

Medicinal plants are vital for basic healthcare in many countries, especially developing countries, as they have long been used as traditional medicine for a range of ailments (Ezeigwe et al., 2020). Medicinal plants include a wide range of bioactive compounds, such as alkaloids, terpenoids, and flavonoids. These phytochemicals have a range of biological activities, such as anti-inflammatory, antibacterial, antioxidant, and anticancer properties, according to Malekmohammad et al. (2020). For instance, the bark of the Pacific yew tree (*Taxus brevifolia*) is used to extract the anticancer drug paclitaxel. Plants are the source of some conventional drugs (Cragg & Newman, 2013). Plants have been used to offer many basic human needs, including food, clothing, and shelter, in addition to their medicinal properties (Gurib-Fakim, 2006). Furthermore, innovative alternatives to conventional drug-therapeutic regimens have been made possible by natural compounds derived from medicinal plants. As per Ly et al. (2015), specific medicinal plants have been employed for the treatment and management of illnesses associated with reproduction.

One species of plant in the Asteraceae family is *Ereromastax polysperma*. In Nigeria, we call it "edemididuo." It is found in tropical and subtropical regions of Africa, particularly in Nigeria, Ghana, and Cameroon. In Akwa Ibom (Ibibio), it's called edemididuo, which means purple bark; Yoruba calls it Oyun, Hausa calls it Esinyin, and Igbo calls it Akwukwo. In addition to being widely utilized in traditional medicine to treat a variety of ailments, recent studies have suggested that the herb may potentially have pharmacological qualities. The herb contains antibacterial, anti-inflammatory, wound-healing, and antioxidant properties (Ojewole, 2005). These qualities make it a good choice for the development of innovative drugs meant to cure a variety of diseases. The plants are particularly common in southern Nigeria, where many people think these species can cure internal heat, diabetes, anemia, and infertility (Pandey, 2006). Sometimes, locals call them "blood tonics." Research on these species' impacts on antioxidant health, as reported by Uyoh et al. (2013), offers empirical evidence in favor of their potential as therapeutics.

Methanol extract of *Ereromastax polysperma* has also been reported to have therapeutic potential in the management of certain diseases such as diabetes (Ibikunle *et al.*, 2017). However, the safety of administration of the extract has not been fully evaluated, particularly with respect to its effects on hematological parameters. This study aims to assess the phytochemical constituents of methanol extract of *Ereromastax polysperma* leaves and its effect on the hematological indices in albino rats.

MATERIALS AND METHODS

Experimental Animals

Sixteen Swiss albino rats were obtained from the animal holding facility of the Faculty of Pharmacy, University of Uyo, Nigeria, and acclimatized for two weeks before the start of the experiment. They were allowed access to water and feed *ad libitum*. All procedures involving animals in this study conformed to the guiding principles in the care and use of animals and the institution's code of ethics for the use of laboratory animals.



Figure 1. Animal Cage. Source: (University of Uyo animal house).

Plant Collection

Fresh leaves of *Eremomatax polysperma* was obtained at the medicinal farm. They were identified and authenticated by the Department of Botany, University of Uyo, Nigeria. The plant material was chopped, dried, and pulverized into a coarse form.



Figure 2. Fresh and Dried *Eremomatax polysperma*.

Plant Extraction

The maceration method was used for the plant extraction. 800g of the dried plant material was weighed using a beam balance. The weighed plant was then transferred into a glass extraction jar. 7.5L/ 750ml of 99.8% methanol in a glass extraction jar and kept at room temperature (25°C - 32°C) for 72 hours (3 days). At the end of 72 hours, the extract was filtered and concentrated to dryness at 40°C using a rotary evaporator. The concentrated methanol extract was stored in a refrigerator at -4°C until further use.

Repeated Graded Dose Administration Study

The extract was administered orally to the animals based on their body weight using a cannula. The administration was given daily to the rats for 14 days and the weight was taken at 48hours intervals. Water was administered in the same manner to the fourth group and this served as a control. On day 15, the animals were made inactive by inhumane method. The animals were cut open and blood was collected from the heart using a needle and 5 ml syringe into ethylene diamine tetra acetic acid (EDTA) specimen bottles. All extracts dosage was determined after toxicity test (LD50).

Blood Collection

Each animal was humanely sacrificed by chloroform inhalation. The rats were anesthetized using chloroform, dissected, and blood withdrawn from the heart through a technique called cardiac puncture, and the deoxygenated blood was drawn using a 5ml syringe and decanted into an EDTA bottle.

Acute Toxicity Study

The acute toxicity of the extracts was evaluated according to the method of Lorke (1983).

Phytochemical Screening

Test for Saponin

Five ml of water was added to the extract and shaken vigorously. It was then allowed to settle for 10-15 minutes. Frothing was observed which therefore signifies the presence of saponin (Milkyas *et al.*, 2016).

Test for Tannins

Five ml of water was added to the extracts and particles were filtered to remove the extracts. 2-3 drops of ferric chloride were then added to the filtrate. A blue-black precipitate was observed indicating the presence of tannins (Milkyas *et al.*, 2016).

Test for flavonoids

Five ml of water was added to the extracts and then placed in a water bath and boiled. After boiling it was removed and filtered. After filtration, the sample was separated into two portions and 2-3 drops of Magnesium metal and conc. HCl was added to one portion. The color

changes to Orange indicating the presence of flavonoids (Milkyas *et al.*, 2016).

Test for Alkaloid

Five ml of 5% HCl was added to the diluted extracts, then the sample was placed in a water bath and allowed to boil. After boiling the sample was filtered and separated into two portions. 2-3 drops of Dragendoff reagent were added to one portion of the sample and kept overnight to determine the positivity or negativity of the sample. An orange ppt. was formed indicating the presence of an Alkaloid (Milkyas *et al.*, 2016).

Test for Cardiac Glycoside

▪ *Salkowski's Test*

A few amounts of extract were dissolved in 5 ml of dichloromethane. 1 ml of concentrated H₂SO₄ was then added gently by running it down the side of the test tube. A reddish-brown was observed indicating the presence of Salkowski's (cardiac glycoside) (Panchal & Parvez, 2019).

▪ *Killer-Killiani test*

The extract was dissolved in glacial acetic acid containing drops of ferric chloride solution. The mixed solution was filtered to remove the particle. 1ml of euphoric acid was added openly. A brown ring forms at the interface indicating the presence of cardiac glycoside (Panchal & Parvez, 2019).

▪ *Lieberman's test*

The extract was dissolved in acetic anhydride and cooled in ice. Sulphuric acid (H₂SO₄) was added to the solution. The color was observed to change from green to blue and brown indicating the presence of a steroidal nucleus (Kumar *et al.*, 2014).

Test for Anthraquinones

▪ *Free Anthraquinones*

0.2g of external was diluted in 5 ml of toluene. The solution was then filtered. 2 ml of 10% ammonia (NH₄OH) was added to the filtered solution. There was

no colour change at the interphase indicating the absence of Anthraquinone (Kumar *et al.*, 2014).

▪ *Combined Anthraquinones*

0.2g of extract was diluted in 5 ml of concentration H₂SO. The solution was then boiled and filtered. 2ml of toluene was added to the filter and shaken. 1 ml of ammonia solution (NH₄OH) was added to the toluene layer and shaken. There was no colour change at the interphase indicating the absence of Anthraquinone.

Determination of Hematological Parameters

Blood was collected from the left ventricle of each animal in a vial containing 0.5 M EDTA. Hematological parameters were determined after day 14 of treatment using an Automated Mindray BC-5300 Hematology Analyzer Made in China at the University of Uyo Teaching Hospital.

Statistical Analysis

The data obtained from the study were subjected to statistical analysis using International Business Machine of Statistical Package for Social Science (IBM SPSS version 25). Data were presented as mean±standard deviation. ANOVA test was used as an inferential statistics and probability less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity Study

Acute toxicity test did not show any mortality, morbidity, or other apparent signs of toxicity at the doses used which indicates that the extract is not toxic at the maximum dose of 5000 mg/kg bw. With this in mind, 1/10th, 2/10th, and 3/10th of this maximum dose (5000 mg/kg) was adopted for the study which gave rise to 500, 1000, and 1500 mg/kg doses of the extract used in the treatment groups. the LD50 5000mg/kg was deduced from the Anex 2d OECD guidelines.

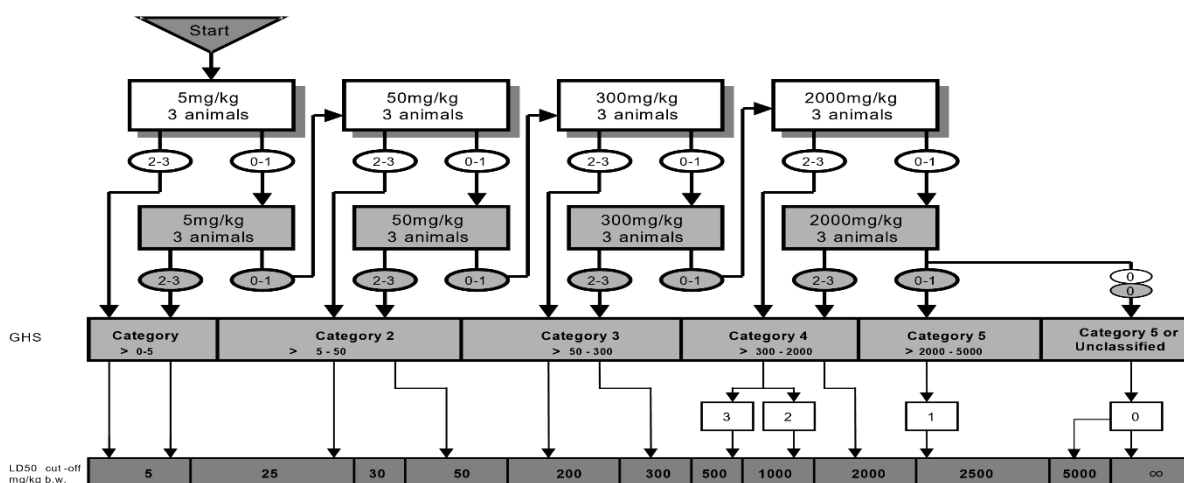


Figure 3. Anex 2d guideline. Source: (OECD 2001)

Phytochemical Screening

The phytochemical screening of crude methanolic extracts of leaf *Eremomatax polysperma* revealed the

presence of some secondary metabolites such as alkaloids, Saponin, Flavonoid, and cardiac glycosides.

Table 1. Phytochemical Screening of leaf of *Eremomatax polysperma*.

Test	Interferences
	Extract
Alkaloids	+
Saponin	+
Tannis	+
Flavoniods	+
Cardiac	
Salkowski's Test	+
Killer-Killiani test	+
Lieberman;s test	+
Anthraquinones	
Free Anthraquinones	-
Combine Anthraquinones	-

+ indicate Presence – indicate Absence

Body Weight

Body weights of albino rats from both the control and treated groups increased progressively throughout the experimental period as shown below;

Table 2. Average weight of the albino rat per treatment.

DAY	1	2	3	4	5	6	7	8
Treatment	Weight(g)	Weight(g)	Weight(g)	Weight(g)	Weight(g)	Weight(g)	Weight(g)	Weight(g)
Control	98 ± 5.48	102.75±5.22	112.25±8.26	106±5.69	112.5±6.85	105.5±6.51	116.75±7.08	118.5±7.67
500mg/kg	94 ± 4.64	109 ± 4.20	107.75±4.09	106.75±4.10	110.25±5.72	106.25±4.71	121.25±6.16	121.75±6.20
1000mg/kg	85 ± 3.76	96.25±4.27	96 ± 4.60	95±4.04	98±5.10	93.5±5.04	107±6.35	110.5±6.41
1500mg/kg	91.75±8.25	102.5± 5.62	104.5±5.95	100.25±5.68	119.5±6.28	116.5±6.41	124.25±6.60	127.75±7.02

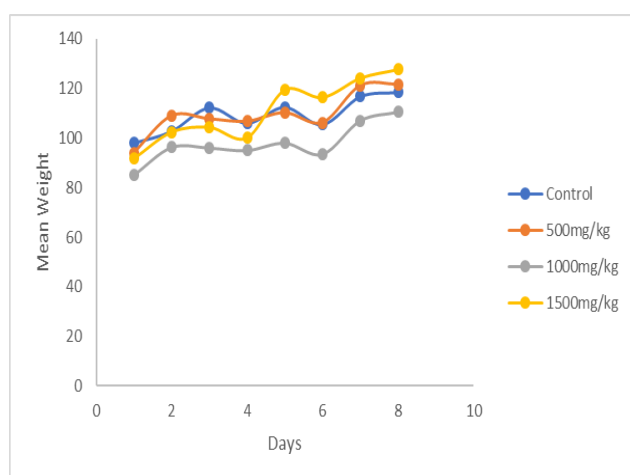


Figure 4. Average weight of the albino rat per treatment.

Hematological Parameters

The provided Table 3 presents the results of a study investigating the effects of different doses (mg/kg) of *Eremomastax polysperma* on various blood parameters. The statistical test employed was ANOVA, with a predetermined significance level of $p < 0.05$ and $p < 0.01$. While the non-significant differences level of $p > 0.05$. The ANOVA analysis revealed statistically significant differences in RBC and HGB, among the control group and the treatment group of 500mg/kg ($p < 0.05$) and also at HCT and the treatment group 500mg/kg ($p < 0.01$). However, no significant differences were observed for WBC, MCV, Lym, Gran, and Mid percentages. These findings suggest that the administration of 500mg/kg doses of the substance or treatment may have specific effects on the Red blood cells and Hemoglobin of the rat.

Table 3. Hamatological Parameters of control and groups treated with graded doses of methanol leaves extract of *Eremomastax polysperma*.

Dose mg/kg	WBC (10 ³ /μL)	Lym (%)	Gran (%)	Mid (%)	Lym (10 ³ /μL)	Gran (10 ³ /μL)	Mid (10 ³ /μL)	RBC (10 ⁶ /μL)	HGB g/dL	HCT (%)	MCV fL
Control	9.53 ± 1.11	87.2 ± 0.97	3.38 ± 0.60	9.48 ± 0.42	8.33 ± 1.06	0.30 ± 0.03	0.90 ± 0.08	7.08 ± 0.10	14.4 ± 0.24	46.1 ± 0.74	65.10 ± 0.77
500mg/kg	9.61 ± 0.41	87.6 ± 0.80	4.18 ± 0.69	8.23 ± 0.35	8.41 ± 0.30	0.41 ± 0.09	0.79 ± 0.05	6.32 ± 0.19 ^a	12.9 ± 0.24 ^a	42.7 ± 0.52 ^b	67.7 ± 1.24
1000mg/kg	10.49 ± 1.62	79.9 ± 4.92	9.4 ± 3.53	10.7 ± 1.06	8.4 ± 1.48	0.96 ± 0.35	0.87 ± 0.33	6.75 ± 0.08	13.48 ± 0.24	44.3 ± 0.39	65.5 ± 0.62
1500mg/kg	8.71 ± 0.87	84.33 ± 1.11	6.1 ± 0.98	9.63 ± 0.66	7.36 ± 0.75	0.53 ± 0.12	0.83 ± 0.07	6.55 ± 0.24	13.3 ± 0.37	44.95 ± 0.39	68.65 ± 1.92

WBC (white blood cell count), Lym (lymphocytes), Gran (granulocytes), Mid (mid-range cells), RBC (red blood cell count), HGB (hemoglobin), HCT (hematocrit), and MCV (mean corpuscular volume)

Table 4. Hematological Parameters of control and groups treated with graded doses of methanol leaf extract of *Eremomastax polysperma*.

Dose mg/kg	MCH Pg	MCHC g/dl	RDW-CV (%)	RDW-SD fL	PLT (10 ³ /μL)	MPV fL	PDW fL	PCT (%)	P-LCR (%)	P-LCC (10 ⁹ /L)
Control	20.40 ± 0.25	31.3 ± 0.36	17.1 ± 0.37	44.5 ± 1.13	825 ± 32.28	8.10 ± 0.19	10.18 ± 0.45	0.664 ± 0.03	14.70 ± 1.35	121 ± 11.59
500mg/kg	20.43 ± 0.23	30.2 ± 0.21	16.3 ± 0.43	44.2 ± 1.10	794 ± 93.42	7.85 ± .27	9.48 ± 0.40	0.616 ± 0.05	13.58 ± 1.76	103.3 ± 4.44
1000mg/kg	19.93 ± 0.25	30.4 ± 0.30	16.68 ± 0.23	43.7 ± 0.31	862.3 ± 11.25	7.90 ± 0.18	9.8 ± 0.36	0.680 ± 0.01	13.7 ± 1.05	117.30 ± 0.78
1500mg/kg	20.30 ± 0.57	29.5 ± 0.62 ^a	16.68 ± 0.27	46.10 ± 1.48	978 ± 44.96	7.93 ± 0.19	9.53 ± 0.34	0.759 ± 0.04	13.83 ± 1.00	132.30 ± 10.84

mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV and RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet crit (PCT), platelet large cell ratio (P-LCR), and platelet large cell count (P-LCC)

The hematological parameters of treated and control rats are presented in Table 4. The ANOVA revealed statistically significant differences in MCHC among the control group and the treatment group of 1500mg/kg ($p < 0.05$). No significant differences ($P > 0.05$) were observed in mean corpuscular hemoglobin (MCH) and red cell distribution width and other parameters including the red cell distribution width (RDW-CV and RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet crit (PCT), platelet large cell ratio (P-LCR), and platelet large cell count (P-LCC), did not exhibit significant differences between the control and treatment groups. These findings suggest that the methanolic leaf extract of *Eremomastax polysperma* has a selective effect on mean corpuscular hemoglobin concentration (MCHC) at 1500mg/kg without affecting other hematological parameters measured in this study.

DISCUSSION

The crude methanolic extracts of *Eremomastax polysperma* leaves were subjected to phytochemical screening, which revealed the presence of various secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, and tannins (Table 1). These phytochemical compounds have significant medicinal importance.

Alkaloids, for instance, have been known for their potent toxic properties, and several alkaloids derived from medicinal plants exhibit various biological activities, including anti-inflammatory, antimalarial, antimicrobial, cytotoxic, antispasmodic, and pharmacological effects (Augusto *et al.*, 2011; Dua *et al.*, 2013; Benbott *et al.*, 2012; Ameyaw and Duker-Eshun, 2009; Thite *et al.*, 2013). Tannins, as reported by previous research, possess antibacterial, antitumor, and antiviral activities (Kumari & Jain, 2012). They exert their effects by precipitating microbial proteins, rendering nutritional proteins unavailable to the microbes.

Cardiac glycosides, another group of phytochemicals identified in the extract, have been utilized in the treatment of congestive heart failure and cardiac arrhythmia (Vladimir and Ludmila, 2001). These compounds operate by inhibiting the Na⁺/K⁺ pump, thereby increasing the levels of calcium ions available for the contraction of heart muscles. This leads to the recovery of cardiac output and a reduction in heart distension (Banso and Adeyemo, 2006; Aiyelaagbe and Osamudiamen, 2009). The presence of these phytochemical compounds in the leaf extracts of *Eremomastax polysperma* may account for the observed biological activities and justify its use as a traditional medicine by the Ibibio tribe and throughout Akwa Ibom State. Specifically, the alkaloids found in the leaf extract

exhibit bioactivity against Gram-positive bacteria and demonstrate cytotoxic effects against leukemia and HeLa cell lines.

Flavonoids play a crucial role as free radical scavengers and primary antioxidants. Therefore, it is important to investigate the presence of phenolic compounds in plant extracts. Polyphenolic compounds possess an aromatic benzene ring with substituted hydroxyl groups, and they can absorb free radicals and chelate metal ions that could catalyze the formation of reactive oxygen species (ROS) and lipid peroxidation. Flavonoids, in particular, are highly significant in promoting human health and disease prevention. The antioxidant potency of flavonoids depends on their molecular structures, including the position of hydroxyl groups and other chemical features (Rajanandh and Kavitha, 2010). These compounds are commonly found in plants in the form of glycosides, with quercetin being one of the most abundant flavonoids known for its excellent antioxidant properties due to its favorable structural characteristics (Kalita *et al.*, 2013).

Assessment of hematological parameters is a valuable approach for evaluating the harmful effects of toxic agents (Agbaje *et al.*, 2009) and determining an individual's health status (Burtis *et al.*, 2012). Changes in the hematological system can provide insights into the prediction of toxicity in animals (Olson *et al.*, 2000), and alterations in the concentrations of red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are particularly important for diagnosing anemia. These parameters not only indicate the potentially harmful effects of herbal remedies but also shed light on their impact on blood-related conditions.

In the study, administration of the plant extract did result in significant changes in RBC, HCT, and HGB levels compared to the control group. This suggests that the extract is likely to cause abnormalities in hematological parameters, such as bleeding, anemia, or bone marrow suppression, in humans. The significant change in RBC (erythrocyte count) was confirmed by the increased percentage of Hemoglobin in the 500mg/kg-dose recipient group. In normal circumstances, local tissue anoxia leads to the formation of a glycoprotein called erythropoietin, which stimulates increased production of erythrocytes (Bowman & Rand, 1980). *Eremomastax polysperma* leaf extract likely contains erythropoietin-like agent(s) which is/are responsible for the increased production of erythrocytes. This finding did not align with the report by Arsad *et al.*, (2013), indicating the relatively unsafe nature of the plant extract in terms of hematological effects.

Table 3 shows that there were no significant changes in the white blood cell (WBC) count. WBCs, along with their differentials such as lymphocytes, serve as indicators of the body's response to toxic substances,

including plants (Adedapo *et al.*, 2004). WBCs and lymphocytes play a crucial role in the body's defense mechanism. The WBC counts in both the control group and the groups treated with doses of 500 mg/kg and 1000 mg/kg body weight exceeded the normal physiological range of 5.00-8.96/mm³ (Mitruka *et al.*, 1977). The percentage values for lymphocytes in the treatment groups were within the normal physiological range of 65.00-84.50%, except for the control and 500 mg/kg group.

Table 4 shows that there was a significant decrease in MCHC at 1500mg/kg- dose. The most common complication of living with low MCHC levels is a lack of energy and decreased stamina. This can limit activities. In severe cases, anemic hypoxia can occur as a result of low MCHC levels. When MCHC levels are very low, the body could struggle to provide enough oxygen to all its tissues. This therefore signifies that the extract of *Eremomastax polysperma* at a higher dose is not good for the body. This study was not in line with the research of Ramadan & Alshamrani (2015) who had no significant difference in MCHC.

CONCLUSION

The acute toxicity test did not show any mortality, morbidity, or other apparent signs of toxicity at the doses used which indicates that the extract is not toxic at the maximum dose of 5000 mg/kg bw. With this in mind, 1/10th, 2/10th, and 3/10th of the maximum dose (5000 mg/kg) was adopted for the study which gave rise to 500, 1000, and 1500 mg/kg doses of the extract used in the treatment groups. From the result, the body weights of experimental animals 1/14*5000 from both the control and treated groups increased progressively throughout the experimental period. Also, phytochemical screening of the extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, and, cardiac glycosides. The hematological evaluation revealed that the administration of the plant extract brought about a significant difference in RBC, HGB, and HCT. Also, mean corpuscular hemoglobin concentration (MCHC) showed a significant decrease with increasing doses of the extract, indicating an adverse effect on the body. Other parameters, including WBC, MCV, PLT, MPV, MCH, Lym, Gran, Mid, RDW-CV, RDW-SD, PLT, PDW, PCT, P-LCR, and P-LCC did not exhibit significant differences between the control and treatment groups. This suggests that the extract is likely to cause abnormalities in hematological parameters, such as bleeding, anemia, or bone marrow suppression in humans.

Recommendation

Based on the findings of this study, the following recommendations can be made:

- Further investigation: Conduct additional studies to identify and isolate specific bioactive compounds from the crude methanol extract of *Eremomastax polysperma*. These isolated compounds can be subjected to further pharmacological and toxicological studies to determine their therapeutic potential and safety profiles.
- Clinical trials: Perform clinical trials to evaluate the efficacy and safety of *Eremomastax polysperma* extracts or isolated compounds in humans, particularly in the treatment of specific conditions such as inflammation, malaria, and bacterial infections.

Competing Interest: The authors declare that there is no competing interest.

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