ACUTE TOXICITY AND EFFECT OF *Terminalia mantaly* H. Perrier (COMBRETACEAE) LEAF EXTRACT ON HAEMATOLOGICAL PARAMETERS OF *WISTAR* RATS

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BENIN CITY, EDO STATE

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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CERTIFICATION

This is to certify that this project was carried out by OSAGUONA EGHOSA PRECIOUS, with Matriculation Number, PHA1908581, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

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DEDICATION

This work is foremost dedicated to God Almighty, for His infinite mercy, guidance, and sustenance throughout my academic journey in the School of Pharmacy.

It is also lovingly dedicated to my family: My Father, Mother, and Siblings. Your immense love, unwavering support, financial sacrifices, and constant guidance have been my foundation and inspiration.

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ABSTRACT

The leaf of *T. mantaly* is a component of traditional medicine, yet its scientific safety profile, particularly on the blood, is not well-established. This research aimed to provide information on the acute toxicity profile and a comprehensive description of the haematological effects of the plant's methanol leaf extract in *Wistar* rats, by examining both the immediate effect of a single high dose, and the sub – acute effect of repeated daily administrations.

The leaf of *T. mantaly* was collected, authenticated, dried and milled into powder form. The powder (1050 g) was Soxhlet extracted using methanol, concentrated and dried to obtain 27.98% yield of extract. Acute toxicity test was done using Lorke's method. Evaluation of the acute effect of a single high dose of 5000 mg/kg and the sub – acute effect of graded doses (200, 400 and 800 mg/kg) of the extract on haematological parameters of female *Wistar* rats, were done using standard methods.

The acute toxicity test revealed that the extract is relatively safe with $LD_{50} > 5000$ mg/kg. However, administration of the 5000 mg/kg dose of the extract revealed a non – toxic physiological stress response, which manifested as a decrease in platelets (thrombocytopenia) and lymphocytes (lymphopenia), as well as a sharp rise in neutrophils (neutrophilia). The 28 days sub – acute study demonstrated the safety profile associated with the administration of lower graded doses of the extract. An immunomodulatory effect, with a slight increase in the number of lymphocytes and platelets, were observed.

In conclusion, the methanol leaf extract of *T. mantaly* possesses a high margin of safety and it is relatively safe, at the tested doses. However, caution is seriously advised in its use, at high doses.

CHAPTER ONE

1.0 Introduction

Terminalia mantaly, a tree indigenous to Africa, is increasingly recognized for its diverse medicinal, ecological, and industrial applications. Traditionally, various parts of the plant have been used to treat ailments such as wounds, ulcers, malaria, and infections, reflecting its importance in African ethnomedicine. Scientific investigations have confirmed the presence of valuable phytochemicals in *T. mantaly*, including alkaloids, flavonoids, saponins, tannins, steroids, triterpenes, and phenols, which contribute to its notable antioxidant, antibacterial, and antiplasmodial activities. Beyond its medicinal uses, *T. mantaly* is valued for its environmental benefits, such as urban heat mitigation due to its dense, evergreen canopy, and its potential as a sustainable resource in industrial applications like scale inhibition and drilling mud formulation. These multifaceted properties make *T. mantaly* a promising subject for further research in pharmaceutical, nutraceutical, and ecological fields, warranting a comprehensive review of its uses and bioactive properties. (Yunusa *et al.*, 2025).

In African ethnomedicine, *T. mantaly* holds a revered status, with its leaves, bark, roots, and fruits utilized for centuries to treat a wide array of ailments. Traditional healers have long harnessed the plant’s therapeutic properties to address conditions such as chronic wounds, skin infections, malaria, and gastrointestinal disorders, reflecting its deep-rooted significance in communities where access to modern healthcare may be limited. These traditional uses are now supported by a growing body of scientific research, which has identified a rich phytochemical profile in the plant, including alkaloids, flavonoids, tannins, saponins, steroids, triterpenes, and phenols. The validation of these traditional practices through modern science highlights the importance of integrating indigenous knowledge with contemporary research to unlock the full medicinal potential of *T. mantaly*. Beyond its medicinal applications, it plays a pivotal role in environmental sustainability. Its broad, evergreen to semi-evergreen canopy provides substantial shade, making it an effective tool for mitigating urban heat islands in rapidly growing African cities. (Adedeji, 2022).

The tree's deep root system contributes to soil stabilization, reducing erosion in regions vulnerable to heavy rainfall or land degradation. Additionally, its adaptability to various ecological conditions supports its use in reforestation and afforestation projects, enhancing biodiversity and aiding in carbon sequestration. In industrial contexts, *T. mantaly* shows promise as a sustainable resource, with its extracts being explored for applications such as scale inhibition in pipelines and the formulation of eco-friendly drilling muds for oil exploration. These diverse attributes underscore the tree's potential to address pressing challenges in health, environmental management, and sustainable industry.

The growing interest in *T. mantaly* reflects its unique ability to bridge traditional knowledge with modern innovation. As global demand for natural remedies, climate-resilient urban planning, and sustainable industrial materials continues to rise, this African species offers a wealth of opportunities for interdisciplinary research. A comprehensive understanding of its bioactive compounds, ecological benefits, and industrial applications is essential to fully harness its potential, making *T. mantaly* a compelling subject for further investigation and a valuable asset for addressing contemporary societal and environmental needs. (Manikyam *et. al*., 2025).

1.1 *Terminalia species*

Terminalia is a large, pantropical genus in the family Combretaceae, comprising over 200 – 250 species of trees and shrubs distributed across Africa, Asia, Australia, and the Americas. The genus is taxonomically complex, with species often distinguished by morphological, anatomical, palynological, cytogenetic, and molecular characteristics.

Taxonomic Classification (Zhang *et al*., 2019):

Kingdom: Plantae

Phylum: Angiosperms

Class: Eudicots

Order: Myrtales

Family: Combretaceae

Genus: *Terminalia*

Species: *Terminalia mantaly*

Authority: H. Perrier

1.1.1 **Description**

T. mantaly is a fast-growing, evergreen tree native to Africa, it's notable for its broad, dense canopy and minimal leaf shedding, which provides year-round shade and cooling. The leaves contain a variety of phytochemicals; including alkaloids, flavonoids, saponins, cardiac glycosides, tannins, steroids, triterpenes, and phenols. It demonstrates strong antioxidant and antibacterial activities against several clinical bacterial strains. The tree is highly effective for urban heat mitigation, lowering surface and ambient temperatures beneath its canopy, increasing soil moisture, and maintaining foliage throughout the year, making it valuable for ecological sustainability and urban landscaping. Additionally, it hosts a diverse range of endophytic fungi, which may contribute to its medicinal properties and potential for biotechnological applications. Growing to heights of 10 – 20 meters, the tree's dense, spreading canopy and robust adaptability to diverse soil types and climates, make it a prominent feature in both rural landscapes and urban environments across Africa. Its aesthetic appeal, coupled with its functional benefits, has positioned it as a specie of significant ecological and socioeconomic value, warranting comprehensive exploration of its properties and potential (Manikyam *et al.*, 2025).

1.1.2 **Phytochemical Composition and Bioactivity**:

T. mantaly is increasingly recognized in scientific literature for its rich and complex phytochemical profile, which is the basis for its diverse biological activities (Yunusa et al., 2025). Various parts of the plant, especially the leaves and bark, have been shown to be a potent source of valuable secondary metabolites. Qualitative phytochemical screening has consistently confirmed the presence of several major classes of compounds.

These include alkaloids, flavonoids, saponins, tannins, steroids, triterpenes, and phenols (Yunusa *et al.*, 2025; Manikyam *et al.*, 2025). The presence of these compounds, particularly the high levels of phenols and flavonoids, is directly linked to the plant's notable bioactivity. The biological activities validated in scientific investigations are broad and support many of its traditional uses:

- **Antioxidant Activity:**

  The extracts, particularly methanolic leaf extracts, demonstrate strong antioxidant properties (Yunusa *et al.*, 2025). This activity is significant as it supports the plant's potential use in managing or mitigating diseases related to oxidative stress (Kipré *et al.*, 2023).

- **Antibacterial Activity:**

T. mantaly has shown significant antibacterial efficacy against several important clinical bacterial strains. Specifically, leaf extracts have demonstrated effectiveness against pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* (Yunusa *et al*., 2025). This provides a scientific basis for its traditional use in treating various infections.

- **Insecticidal Potential:**

In addition to its effects on microbes, the plant's phytochemicals also exhibit notable insecticidal properties (Yunusa *et al*., 2025).

- **Cytotoxic and Anticancer Potential**:

Research has moved into more advanced biotechnological applications. Extracts from *T. mantaly* have been successfully used for the "green synthesis" of gold nanoparticles. These biogenic nanoparticles displayed enhanced cytotoxic effects when tested against various cancer cell lines, indicating a promising potential for applications in future cancer therapy (Majoumouo *et al*., 2020).

- **Biotechnological Resource:**

Beyond its direct medicinal action, the plant is also a valuable biotechnological resource. It is known to host a diverse community of endophytic fungi. These fungi are capable of producing a range of industrially relevant enzymes, suggesting that *T. mantaly* can be a source of novel bioactive chemicals and biotechnological tools (Toghueo *et al*., 2017).

1.1.3 Antimalarial and Ethnomedicinal Applications

In African ethnomedicine, *T. mantaly* holds a revered status. For centuries, traditional healers have harnessed the therapeutic properties of its leaves, bark, roots, and fruits to treat a wide array of common and serious ailments. Its applications reflect a deep-rooted significance in communities where it serves as a primary source of healthcare. Common traditional uses include the treatment of chronic wounds, skin infections, gastrointestinal disorders, ulcers, and, most notably, malaria (Mbouna *et al.*, 2018). The widespread use of *T. mantaly* for treating malaria, in particular, has prompted significant scientific investigation to validate these traditional practices. This growing body of research has provided strong scientific support for its antiplasmodial (antimalarial) activity:

- ***In-vitro* and *In-vivo* Efficacy:**

  Studies using aqueous stem bark extracts have demonstrated potent efficacy against the malaria parasite. These extracts were shown to be effective both *in-vitro* (against *Plasmodium falciparum*, the most dangerous human malaria parasite) and *in-vivo* (in animal models infected with *Plasmodium berghei*) (Mbouna *et al.*, 2018; Tchatat Tali *et al.*, 2020).

- **High Safety Margin:**

  Critically, these *in-vivo* studies in animal models also revealed that the active extracts possess high safety margins, supporting the plant's traditional use as a well-tolerated remedy (Mbouna *et al.*, 2018; Tchatat Tali *et al.*, 2020).

- **Mechanism of Action:**

  More advanced research has begun to pinpoint the mechanism of action. Chromatographic sub-fractions isolated from the plant's extracts were found to potently inhibit multiple crucial stages of the parasite's lifecycle, including the rings, merozoite egress (release from red blood cells), and invasion of new cells (Mbouna *et al.*, 2021).

- **Isolation of Active Compounds**:

  Researchers have successfully isolated specific active antimalarial agents from the plant. Key phytometabolites identified in active fractions include D-limonene and caryophyllene (Mbouna *et al.*, 2021). Furthermore, specific triterpenoid compounds, such as arjunglucoside I and arjungenin, have been isolated and confirmed as active antimalarial principles (Samuel and Adekunle, 2021). This validation of traditional knowledge through modern science highlights the importance of T. mantaly as a source for new antimalarial drugs and underscores the value of integrating indigenous knowledge with contemporary research (Samuel and Adekunle, 2021).

1.1.4 **Biotechnological and Pharmaceutical Potential**

The pharmaceutical and biotechnological potential of *T. mantaly* is a rapidly expanding field of research, driven by the plant's rich phytochemical profile. Its traditional use in treating ailments like malaria and infections has provided a strong basis for modern scientific investigation, which has begun to validate and even expand upon these applications. A significant area of its pharmaceutical potential lies in its antimalarial properties.

Scientific studies have validated its ethnomedicinal use for malaria treatment. Aqueous stem bark extracts have demonstrated potent in-vitro and in-vivo efficacy against *Plasmodium falciparum* and *Plasmodium berghei*, respectively. These extracts not only show high antiplasmodial activity but also high safety margins in animal models (Mbouna *et al*., 2018; Tchatat Tali *et al*., 2020).

1.1.5 **Environmental and Urban Applications**

Beyond its medicinal and pharmaceutical uses, *T. mantaly* is highly valued for its significant environmental and industrial applications. Its unique physical characteristics make it a pivotal species for addressing modern urban and ecological challenges. The most prominent application is in urban heat mitigation. In rapidly growing African cities, *T. mantaly* is an effective tool for mitigating urban heat islands (Adedeji, 2022). This is due to its dense, broad, and evergreen to semi-evergreen canopy, which provides substantial and year-round shade. Scientific investigations have confirmed its high effectiveness in urban cooling; it measurably lowers both surface and ambient temperatures beneath its canopy. In addition to cooling, its dense foliage also increases soil moisture and enhances relative humidity, further contributing to a more comfortable microclimate (Manikyam *et al*., 2025). Its aesthetic appeal and robust adaptability to diverse urban conditions make it a preferred species for urban landscaping and climate resilience strategies. The tree also plays a crucial role in environmental sustainability and land management. Its deep root system is highly effective for soil stabilization, which helps to reduce erosion in regions vulnerable to land degradation or heavy rainfall. Its robust adaptability to various ecological conditions and soil types makes it an excellent candidate for reforestation and afforestation projects. By being used in this way, it helps to enhance local biodiversity and aids in carbon sequestration. In an industrial context, T. mantaly shows promise as a source of sustainable, eco-friendly materials. Its extracts are being explored for novel applications, such as a scale inhibitor in pipelines and as a component in the formulation of environmentally friendly drilling muds for oil exploration. This multifaceted nature, bridging ecological benefits with industrial potential, underscores the tree's value as a key sustainable resource (Manikyam *et al*., 2025).

Figure 1: *T. mantaly* tree growing in its cultivated habitat in the premises of the Faculty of Pharmacy (New site), University of Benin, Benin City.

1.2 Haematological Parameters

Haematological parameters, also known as blood parameters or haematology indices, refer to the measurable components and characteristics of blood that provide insights into an individual's health status, particularly related to blood cell production, function, and disorders. These parameters are typically assessed through tests like the complete blood count (CBC) or full blood count (FBC), which evaluate red blood cells (RBCs), white blood cells (WBCs), platelets, and related metrics. They help diagnose conditions such as anaemia, infections, leukemias, clotting disorders, and inflammatory diseases (Adekunle, *et al*. 2021).

Some important haematological parameters are shown in Table 1 below.

Table 1: Normal range values of some clinical parameters for Rats and Rabbits that are commonly used in research (Ozolua and Bafor, 2019).

Parameter	**Definition**	**Normal Range (Approximate value) Rats**	**Normal Range (Approximate value) Rabbits**
White Blood Cell (WBC) Count	Total number of white blood cells, involved in immune response and fighting infections	3.0 – 17.0 × 10^3/µL varies slightly by gender and age.	4.0 – 13.0 × 10^3/µL
Red Blood Cell (RBC) Count	Number of circulating red blood cells, essential for oxygen transport.	5.0 – 10.0 × 10^6/µL	4.0 – 8.0 × 10^6/µL
Haemoglobin (Hb)	Protein in RBCs that binds oxygen; measures concentration in blood.	11.0 – 19.0 g/L.	11.1 – 15.6 g/L.

Haematocrit (HCT) or Packed Cell Volume (PCV	Proportion of blood volume occupied by RBCs.	35 - 57%	30 - 50%
Mean Corpuscular Volume (MCV)	Average size of RBCs; part of RBC indices.	48 – 77 fL	50 - 75 fL
Mean Corpuscular Haemoglobin Concentration (MCHC)	Average haemoglobin concentration in RBCs.	29 – 37 g/dL.	31 – 34 g/dL
Platelet Count	Number of platelets, crucial for clotting.	200 – 1500 × 10^3/μL.	290 – 650 × 10^3/μL
Mean Platelet Volume (MPV)	Average size of platelets.	4.0 – 7.2 fL.	5.17 – 6.06 fL

1.2.1 **Factors affecting haematological parameters**

Haematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), and platelet count, are influenced by a wide array of genetic, physiological, environmental, lifestyle, pathological, and other factors. These influences can alter blood cell production, function, and composition, leading to variations that are crucial for interpreting clinical results accurately. Establishing population-specific reference intervals is essential, as deviations may indicate health issues or normal physiological adaptations. Below are these factors categorically, with explanations, examples, and their impacts on specific parameters. (Li. *et al.*, 2019)

1. **Genetic Factors**:

   Genetic background, including ethnicity, breed (in animals), and genotype, plays a fundamental role in baseline haematological values by affecting cellular morphology, haemoglobin synthesis, and immune responses. These factors can lead to inherent variations that are not pathological but must be accounted for in reference ranges. (Ambayya *et al.*, 2023). Different ethnic groups exhibit variations due to genetic polymorphisms. For instance, benign neutropenia is more prevalent in Arab populations (12.8%) compared to Western groups (e.g., 0.79% in White Americans), potentially lowering WBC counts without disease. In Saudi populations, MCV and Hb levels are lower than in Caucasians but similar to some African groups, possibly linked to genetic adaptations or dietary influences. Genetic differences can also influence RBC indices; for example, certain genotypes in animals show higher PCV and WBC, impacting oxygen transport and immunity (Nseabasi Etim, 2014).

2. **Physiological Factors**:

Physiological states related to age, sex, pregnancy, and circadian rhythms cause predictable changes in haematological parameters due to hormonal, developmental, or metabolic shifts (Borghesan *et al.*, 2016).

- **Age**:

Parameters like RBC, Hb, and HCT often peak in young adults (e.g., higher in 30 – 39 years compared to 18 – 29 or ≥ 40 years) and decline with advancing age due to reduced hematopoietic stem cell reserve. Platelet count also decreases with age. In animals, older groups show higher PCV and WBC, indicating maturation effects on oxygen-carrying capacity and immunity. For example, in goats and sheep, age significantly raises RBC, Hb, PCV, MCV, and MCHC, enhancing oxygen transport in adults (Alsalem *et al.*, 2022)

- **Sex/Gender**:

Males typically have higher RBC (5.4 vs. 4.5 × 10^{12}/L), Hb (14.9 vs. 12.6 g/L), HCT, MCV, and MCHC due to androgen effects on erythropoiesis, while females have higher platelet counts (290 vs. 254 × 10^{9}/L), possibly from estrogen and menstrual iron loss. Anaemia prevalence is higher in women (22.5% vs. men), linked to lower iron stores. In animals, males often show higher PCV and RBC, while females have elevated WBC and MCHC, affecting immune and clotting functions. (Addass *et al.*, 2012, Anosa *et al.*, 2025).

- **Pregnancy**:

Pregnancy induces changes like increased plasma volume, lowering HCT and Hb, and altering WBC due to immune modulation. In marmosets, pregnancy affects parameters like blood urea nitrogen and total protein, necessitating specific reference intervals. This can mimic dilutional anaemia, impacting oxygen delivery. (N.N. *et al.*, 2014).

3. **Environmental Factors**:

Environmental conditions, including altitude, geography, season, and colony/source (in animals), affect parameters through adaptive responses like hypoxia or temperature changes.

- **Altitude and Geography**:

Higher altitudes increase RBC, Hb, and HCT due to hypoxia-induced erythropoiesis. Regional differences in Saudi Arabia show higher RBC and Hb in central regions, with higher anaemia in eastern areas possibly from sickle cell prevalence (Kruska *et al*., 2022)

- **Neutropenia rates:**

Neutropenia rates rise at higher elevations, lowering WBC.

- **Seasonal Patterns and Climate**:

Seasonal variations can alter parameters. For example, traditional husbandry in varying climates lowers overall values compared to controlled environments.

- **Colony/Source:**

In marmosets, different colony origins affect parameters like alkaline phosphatase and serum albumin with medium/large effect sizes, due to environmental or genetic drift.

1.2.2 Why are Haematological parameters biomarkers for toxicity?

Haematological parameters are widely used as biomarkers for toxicity because they provide sensitive, early, and integrative indicators of physiological disturbances caused by toxic substances (Kim, 2021).

Mechanisms Linking Haematological Changes to Toxicity

- **Direct Impact on Blood Cells**:

Toxicants can damage or alter the production, morphology, and function of blood cells (e.g., red blood cells, white blood cells, platelets), leading to measurable changes such as anaemia, leukocytosis, or thrombocytopenia.

- **Disruption of Haematopoiesis**:

Many toxicants interfere with bone marrow function, affecting the synthesis and maturation of blood cells, which is reflected in altered haematological profiles.

- **Oxidative Stress and Immune Response:**

Toxic exposures often induce oxidative stress and immune responses, which can be detected through changes in haematological parameters (e.g., increased white blood cell count, altered lymphocyte and neutrophil ratios) (Ramesh, 2023).

- **Organ Dysfunction**:

Toxicity to organs like the liver or kidney can indirectly affect blood parameters due to impaired metabolism, filtration, or synthesis of blood components. (Petterino and Argentino-Storino, 2006).

1.3. **Toxicity**

Toxicity refers to the degree to which a substance can cause harm to a living organism. It encompasses all adverse effects that a chemical, drug, or natural product may produce, ranging from mild irritation to severe organ damage or death. It is an important factor to take into account while evaluating therapeutic plants. To determine appropriate dosage limits for medicinal usage and to ascertain the safety profile of plant extracts, toxicity studies are crucial. Toxicity can be evaluated through various tests to determine the potential risks associated with exposure. Based on the length of time and frequency of the exposure, these investigations are usually divided into four categories: acute, sub-acute, sub-chronic and chronic toxicity. However, this research work was focused on acute and sub-acute toxicity study.

1.3.1 **Acute Toxicity**

Acute toxicity is the adverse effect(s) that occur within a short period after a single dose or multiple doses given within 24 hours. Acute toxicity studies are typically performed at the beginning of substance evaluation and are designed to identify symptoms, target organs, and the extent of harm caused by a single exposure. The outcome is often expressed as the LD_{50} (lethal dose for 50% of the test population).

1.3.2 **Sub-acute Toxicity**

Subacute toxicity refers to the adverse effects resulting from repeated exposure to a substance over a short period, usually up to 28 days. Subacute toxicity studies involve daily administration of the test substance and are used to assess cumulative effects, target organ toxicity, and physiological or metabolic changes that may not be apparent in acute studies. These studies help predict the long-term safety of a compound.

1.4 **Why Rats?**

It's because of their physiological resemblance to humans and their longstanding application in toxicity assessment. Rats make excellent study subjects, in the assessment of toxicity of various substances.

1.4.1 **Acclimatization**

This term refers to the process/period during which newly arrived research animals are allowed to fully recover from shipping and adjust to new surroundings, food, light/dark cycles, cage/pen mates and personnel prior to being used in research, teaching, or testing protocols.

1.5 **Methanol as solvent for extraction**

Methanol is generally used for extraction processes as due to the following reasons stated below:

1.5.1 Advantages of using methanol as solvent for extraction

1. High Extraction Efficiency: Methanol often yields higher extraction rates for phenolics, flavonoids, alkaloids, and terpenoids compared to other solvents.
2. Strong Antioxidant and Bioactivity Recovery: Extracts obtained with methanol frequently show high antioxidant and anti-inflammatory activities.
3. Low Cost and Availability: Methanol is relatively inexpensive and widely available for laboratory and industrial use.
4. Rapid Extraction: Its volatility and polarity enable faster extraction processes.
5. Broad Solubility: It dissolves a wide range of polar and some non-polar compounds, making it versatile for extracting diverse phytochemicals.

1.5.2 Disadvantages of using methanol as solvent for extraction

1. Toxicity: Methanol is highly toxic to humans and can cause cytotoxic effects if residues remain in extracts.
2. Safety Concerns: It is flammable and poses health and environmental hazards during handling and disposal.
3. Residue Removal Required: Complete removal of methanol from extracts is necessary to ensure safety. This thus increases the processing steps.
4. Not Suitable for Food/Pharmaceutical Use Without Further Purification: Due to its toxicity, methanol-extracted products require rigorous purification before use in consumables.
5. Potential for Co-extraction of Undesired Compounds: Methanol may extract unwanted impurities along with target compounds, affecting extract purity.

1.6. Aim and Objectives

This study was carried out so as to obtain data that could be used to fill the knowledge gap that exists on the toxicological consequence (if any), of administering methanol leaf extract of the plant to *Wistar* rats. From literature, limited data was available on the toxicity profile of *T. mantaly*. Hence, *Wistar* rats were administered the methanol leaf extract (both acutely and sub-acutely) in order to assess the safety profile of the extract, on haematological parameters.

1.6.1 Specific Objectives

Specific Objectives were to;

1. Determine the LD_{50} of the extract.
2. Determine the acute effect of the extract, when administered as a single dose on blood parameters.
3. Determine the sub-acute effect of the extract, when administered daily for 28 days, on haematological parameters.

1.6.2 Significance of Study

This study will help clarify the toxicological safety (on haematological parameters) of the plant and its potential risk (if any), to animal or human health.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Site of the experiment

Following globally recognized guidelines for the use of laboratory animals, this experiment was conducted in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

2.2 Materials

Some materials used in this study are as follows:

Electric and manual weighing balances, Soxhlet apparatus, glassware's (stirrer, spatula, measuring cylinders, test tubes, beakers); hot water baths, orogastric tube; 2, 5 and 10 mL syringes; hand gloves; and containment's (Potassium ethylenediamine tetra-acetate tubes). Other materials include Gentian violet for marking the laboratory rats, cotton wool, 2% Tween 80 (polysorbate 80), distilled water, fridge for preserving the herbal extract, a thermostat-controlled hot air oven, evaporating dishes, etc.

2.2.1 Animals used

For this investigation, forty – two (42) female *Wistar* rats (160g - 180g) were employed. They were purchased and allowed two (2) weeks to acclimatize at the Department of Pharmacology's animal house where the temperature was fairly maintained at about 25±2ºC. The animals were housed in plastic cages with wire mesh roofs and beddings made of sawdust. The rats had access to pelleted feed (Premier feed Mills Co. Ltd.) and water *ad libitum*. Ethical approval was sought and obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City; and the experiment was conducted in compliance with international regulations governing the use of animals.

2.2.2 Plant used

The leaves of *T. mantaly* were collected in the month of April, 2025 from the premises of the Faculty of Pharmacy, University of Benin, Benin City, Edo State. The leaves were rinsed in water to remove dust, then air-dried under shade for a week, transferred to an oven maintained at 60ºC for 45 minutes and then, milled with the aid of an electric miller. The powder obtained was stored in an airtight container.

2.3 Methods

2.3.1 Preparation of Herbal extract

The powdered leaf of *T. mantaly* (1050 g) was Soxhlet extracted (65ºC) using methanol (100%) to obtain the methanol extract. Four hundred grammes (400 g) of the leaf was initially placed into the thimble, and exhaustively extracted. After which successive 400 and 250 grammes of the powdered leaves were extracted. The extract was then concentrated using a rotary evaporator. The residual solvent was completely removed using previously weighed evaporating dishes on a thermostatically controlled water bath maintained at 65ºC. The percentage yield of extract obtained was 293.7 g (27.98%). The dried extract was then stored in a fridge until it was needed.

2.3.2 Toxicity Study

This includes acute and sub-acute toxicity studies.

2.3.2.1 Acute Toxicity Study

a. Observation for any untoward effects/Death:

The Lorke's method of 1983 was used. A total of twelve (12) *Wistar* rats were used, with weights ranging from 160g – 180g. It involved two (2) stages of A and B. In stage A, nine (9) female rats were shared into groups A, B and C; with each group having three (3) rats each. Groups A, B and C were administered 10, 100 and 1000 mg/kg of the extract. They were observed continuously for the first 4 hours for any signs of toxicity; then for 24 hours, and daily for two (2) weeks.

Stage B involved three (3) rats shared into three (3) groups of one (1) rat each. The first group received 1600 mg/kg; second group (2900 mg/kg) and third group (5000 mg/kg body weight of the extract). They were observed continuously for the first 4 hours (as in stage A), then for 24 hours and 14 days respectively, for any signs of toxicity and death.

b. Effect on haematological parameters:

Here, a total of ten (10) female *Wistar* rats were used. The rats weighing between 160g – 180g were fasted overnight. The rats were then shared into two groups (Group A and B) of five (5) rats each. Group A was given 10 mL/kg of 2% Tween-80 solution as control, while group B was administered orally with a single dose of 5000 mg/kg body weight of extract. Three (3) hours post administration of the extract, the animals were sacrificed via chloroform anaesthesia, and blood (3 mL) collected via cardiac puncture into K – EDTA tubes. This was used for assay of haematological parameters.

2.3.2.2 Sub Acute Toxicity Study

Here, a total of twenty (20) female *Wistar* rats were used. The rats (160 – 180g) were shared into four groups (A – D) of five rats each. Group A was given 10 mL/kg of 2% Tween-80 solution as control; while Groups B, C and D were given 200, 400 and 800 mg/kg of extract respectively. For twenty – eight (28) days, all dosages were administered orally, using an orogastric tube, on a daily basis.

2.3.3 Specimen Collection

The rats were anaesthetized in a chamber saturated with chloroform on day 29, and following their removal from the jar, the rats were dissected. Blood samples were obtained via cardiac puncture using 10 mL syringes. The blood samples (3 mL each) were placed in tubes containing drops of potassium tetra-acetate (K-EDTA) and used for haematological analysis, to assay various haematological parameters.

2.3.3.1 Haematological analysis

The automated haematology system, Systemex haematology coagulation system (Model KK2IN Systemex – Incorporation, Kobe, Japan) was used for the haematological analysis at the Department of Haematology, University of Benin Teaching Hospital, Benin City, Edo State. The following parameters analyzed in the collected blood samples were: White Blood Cell (WBC), Red Blood Cell count (RBC), Haemoglobin (HGB), Haematocrit (HCT), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT).

2.4. Statistical analysis

The software used was Graph Pad Instant Version 2.0.5 (UK). Values were expressed as Mean ± Standard Error of Mean (SEM). Paired T – test and One-way analysis of variance (ANOVA) followed by Dunnett comparison tests were used for statistical analysis. Any p – value below 0.05 ($p < 0.05$) was deemed to be significantly different from control.

CHAPTER THREE

3.0 RESULTS

3.1 Acute Toxicity Study:

a. Observation of untoward effects/Death:

Upon administration of the extract to the animals, only reduced movement was observed in the animals that received the 2900 and 5000 mg/kg doses of the extract, when observed for the first four (4) hours. No death (mortality) was observed in any of the groups.

Therefore, LD_{50} was found to be greater than 5000 mg/kg. Also, for the two (2) weeks period the rats were observed, no symptoms of toxicity and death, were observed.

b. Effects on haematological parameters:

The effect of administration of a high dose (5000 mg/kg) of the extract on the haematological parameters of *Wistar* rats are seen in Table 2.

Only the PLT, MPV, PDW and PCT were found to be significantly different from that of the control group.

Table 2: Effect of the 5000 mg/kg dose of *T. mantaly* extract on haematological parameters of female *Wistar* rats

Parameters (Units)	**Control (10 mL/kg)**	**Extract (5000 mg/kg)**
WBC (10^3/µL)	9.71 ± 0.84	8.04 ± 1.50
RBC (10^6/µL)	6.49 ± 0.13	6.38 ± 0.54
HGB (g/dL)	13.49 ± 0.21	12.84 ± 1.09
HCT (%)	37.10 ± 0.58	33.90 ± 2.77
PLT (10^3/µL)	711.2 ± 59.20	567.4 ± 62.73*
MPV (fL)	7.38 ± 0.17	6.62 ± 0.02*
PDW (fL)	15.98 ± 0.16	6.60 ± 0.10**
PCT (%)	0.53 ± 0.02	0.38 ± 0.04*

Key: Values are expressed as Mean ± SEM. n = 5. WBC = White blood cell Count; RBC = Red blood cell Count; HBG = Haemoglobin Count; HCT = Haematocrit; PLT = Platelet Count; MPV = Mean Platelet Volume; PDW = Platelet distribution width and PCT = Plateletocrit.

3.2 Sub – acute toxicity test:

The results obtained after daily administration of graded doses of the methanol leaf extract of *T. mantaly* to female *Wistar* rats for twenty – eight (28) days is as shown in Table 3.

Here, the values for the various haematological parameters assayed in the extract treated group were not significantly different ($p > 0.05$) from that of the control group.

Table 3: Effect of graded doses of *T. mantaly* extract on haematological parameters of female *Wistar* rats.

Parameters (Units)	**Control (10 mL/kg)**	**Extract (200 mg/kg)**	**Extract (400 mg/kg)**	**Extract (800 mg/kg)**
WBC (10^3/μL)	9.83 ± 0.95	11.34 ± 0.65	11.74 ± 0.25	9.73 ± 1.02
RBC (10^6/μL)	6.46 ± 0.13	6.27 ± 0.09	6.16 ± 0.20	6.39 ± 0.17
HGB (g/dL)	13.68 ± 0.23	13.40 ± 0.22	13.40 ± 0.34	13.68 ± 0.28
HCT (%)	37.04 ± 0.63	35.70 ± 0.46	35.86 ± 0.90	35.56 ± 0.70
PLT (10^3/μL)	704.4 ± 58.88	824.80 ± 64.9	765.00 ± 37.7	725.80 ± 40.4
MPV (fL)	7.40 ± 0.19	7.20 ± 0.15	7.04 ± 0.08	7.20 ± 0.22
PDW (fL)	15.86 ± 0.15	15.64 ± 0.04	15.72 ± 0.05	15.62 ± 0.05
PCT (%)	0.52 ± 0.04	0.59 ± 0.04	0.54 ± 0.02	0.52 ± 0.02

Key: Values are expressed as Mean ± SEM. n = 5. WBC = White blood cell Count; RBC = Red blood cell Count; HBG = Haemoglobin Count; HCT = Haematocrit; PLT = Platelet Count; MPV = Mean Platelet Volume; PDW = Platelet distribution width and PCT = Plateletocrit

CHAPTER FOUR

4.0 DISCUSSION AND CONLUSION

4.1 DISCUSSION

General overview of Haematological parameters:

Haematological parameters, also known as blood parameters, are the measurable components and characteristics of blood that offer critical insights into an individual's health status. They are fundamental in diagnostics because they provide sensitive, early, and integrative indicators of physiological disturbances, making them excellent biomarkers for toxicity (Kim, 2021).

Therefore, evaluating haematological parameters is essential for determining the safety profile of any new compound, including herbal extracts (Petterino and Argentino-Storino, 2006). These tests, often part of a complete blood count (CBC), evaluate the production, function, and characteristics of blood cells.

The White Blood Cell (WBC) count is a primary measure of the body's immune system, quantifying the total number of cells involved in fighting infections. A high WBC count, known as leukocytosis, often serves as a red flag for an active infection, significant inflammation (such as in rheumatoid arthritis), or even cancers like leukemia. It can also be elevated due to physiological stress or pregnancy. Conversely, a low WBC count, or leukopenia, is equally concerning as it indicates a compromised immune status. This can be caused by conditions that suppress the immune system, such as HIV, certain viral infections, malnutrition, or as a side effect of treatments like chemotherapy (Ozolua and Bafor, 2019).

Equally vital are the parameters related to red blood cells, which are responsible for oxygen transport. The Red Blood Cell (RBC) count itself quantifies the number of circulating erythrocytes, directly reflecting the blood's oxygen-carrying capacity. When this count is abnormally high (erythrocytosis), it could be a sign of dehydration (which concentrates the blood), chronic hypoxia (where the body compensates for low oxygen by making more RBCs), or a bone marrow disorder known as polycythaemia vera. A low RBC count, on the other hand, points towards anaemia, active haemorrhage (blood loss), or other disorders affecting the bone marrow's production line (Adeneye, 2012).

Closely linked to the RBC count is Haemoglobin (HGB), which measures the concentration of the specific oxygen-binding protein found within each red blood cell. This measurement is one of the most common ways to assess anaemia risk. Low haemoglobin is the hallmark of anaemia and can stem from various causes, including iron deficiency, chronic diseases, or genetic disorders like sickle-cell anaemia. High haemoglobin levels are typically seen in the same conditions that raise the RBC count, such as polycythaemia or dehydration.

The Haematocrit (HCT), also referred to as Packed Cell Volume (PCV), complements the RBC and HGB measurements by determining the proportion or percentage of the total blood volume that is composed of red blood cells. This value is a key indicator of both red blood cell mass and the body's overall hydration status. A high haematocrit can be a sign of dehydration, COPD, or polycythaemia. A low haematocrit indicates a deficit in red blood cells, commonly pointing to anaemia, blood loss, nutritional deficiencies, or leukemia.

Moving away from oxygen transport, the thrombocyte, or platelet, parameters are essential for assessing blood clotting. The Platelet (PLT) count measures the number of these small cell fragments, which are the first responders to injury to form a clot. This count is a direct assessment of the body's risk of bleeding or clotting. A high platelet count, or thrombocytosis, can be caused by infections, chronic inflammation, or certain cancers. It can also be a "reactive" or secondary response, where the body is stimulated (perhaps by immunomodulatory compounds in an extract) to produce more platelets. A low platelet count, or thrombocytopenia, is dangerous as it increases the risk of uncontrolled bleeding. This can be caused by immune disorders, viral infections like dengue, or as a side effect of chemotherapy. Interestingly, a sudden, acute drop in platelets can also occur during severe systemic stress, not because they are destroyed, but because the spleen "hoards" or sequesters them, pulling them out of circulation (Thakkar and Marwaha, 2024).

The platelet indices provide deeper insight into the platelet population. The Mean Platelet Volume (MPV) measures the average size of the platelets. This value is a subtle but important indicator of platelet production and activity. A high MPV suggests a high turnover rate—new, larger platelets are being rapidly produced and released, which can happen in conditions like immune thrombocytopenia. A low MPV, conversely, can be seen in conditions where production is suppressed, like aplastic anaemia. It can also suggest that in an acute stress event, the spleen is preferentially holding back the larger, more active platelets, leaving only the smaller, older ones in the bloodstream.

The Platelet Distribution Width (PDW) is another index that was analyzed. While not defined in detail, its stability is noted as a positive sign, suggesting that the bone marrow is producing a morphologically normal and uniform population of platelets. A significant decrease in PDW, especially when seen alongside drops in PLT and MPV, can be part of the signature of an acute systemic stress response.

Finally, the Plateletcrit (PCT) measures the total platelet mass within the blood. This value provides a comprehensive measure of the total mass of platelets available for clotting. A significant drop in the PCT, particularly in conjunction with low PLT and MPV counts, is a key indicator of acute thrombocytopenia, such as the kind caused by the spleen sequestering platelets during a major systemic stress event. Together, these eight parameters provide a detailed and interconnected snapshot of the haematological system's health.

This study was meticulously designed to provide a comprehensive toxicological evaluation of the methanol leaf extract of *T. mantaly*. The primary goal was to establish a clear safety profile, which is essential before any therapeutic plant can be recommended for ethnomedicinal use (Adeneye, 2012). To achieve this, the haematological system was chosen as the endpoint because it is exceptionally sensitive to toxic insults and serves as a reliable biomarker of systemic health (Kim, 2021; Petterino and Argentino-Storino, 2006).

Acute Toxicity Study

a. Observation for any untoward effect/Death:

The first objective was to determine the acute toxicity and establish the median lethal dose (LD_{50}) of the extract. This step is fundamental for classifying the substance and establishing its general safety margin. The Lorke's method of 1983 was employed, which involved administering progressively higher doses (10, 100, 1000, 1600, 2900, and 5000 mg/kg) to different groups of rats. The results were definitive; no mortality (death) was observed in any group during the observation period, even at the highest possible test dose of 5000 mg/kg. The only clinical signs noted were reduced movement in the animals that received the 2900 and 5000 mg/kg doses, which resolved without further incident. This establishes the LD_{50} of the methanol leaf extract as greater than 5000 mg/kg. According to established toxicity classification guidelines (Adeneye, 2012), this result places the extract in the safest category, indicating that it is relatively safe and possesses a high margin of safety upon acute ingestion. This finding was the prerequisite for proceeding with further haematological analysis.

b. **Effect on Haematological Parameters (Single 5000 mg/kg Dose)**:

The second objective was to assess the immediate, 3-hour post-administration effect of a single, massive 5000 mg/kg dose. This "limit test" is not designed to find a therapeutic dose but to unmask any potential, immediate, and severe toxic effect at an extreme exposure. The results, shown in Table 2, reveal a clear and significant divergence in how the extract affects different blood cell lines. The most crucial finding for this group was the lack of a statistically significant change in the erythrocyte (red blood cell) parameters, specifically the RBC count, Haemoglobin (HGB), and Haematocrit (HCT).

Furthermore, the total White Blood Cell (WBC) count also remained stable when compared to the control group. This is a profoundly positive safety finding. A significant drop in these parameters would have been a critical red flag for acute haemolysis (widespread destruction of red blood cells) (Adeneye, 2012). The stability of these counts strongly indicates that even at this exceptionally high dose, the extract is not acutely haemolytic, does not cause acute anaemia, and does not trigger an immediate, catastrophic collapse of the immune system.

In sharp contrast, all platelet-related parameters showed a statistically significant decrease. The Platelet (PLT) count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), and Plateletcrit (PCT) all fell significantly. This rapid drop, occurring within just three hours, cannot be due to a failure in platelet production (thrombopoiesis), which is a process that takes several days. The most plausible biological explanation is acute splenic sequestration (Thakkar and Marwaha, 2024). The spleen acts as a dynamic reservoir for up to a third of the body's platelets. It is highly likely that in response to the massive physiological stress of the 5000 mg/kg dose, the spleen acutely "hoarded" or sequestered a large portion of the circulating platelets, causing the count in the blood to plummet. The corresponding decrease in MPV and PDW further supports this, suggesting that the spleen may have preferentially held back the largest, youngest, and most active platelets. This demonstrates that the 5000 mg/kg dose, while not lethal, is far from inert. It is biologically potent enough to induce a powerful, immediate systemic stress response, which manifests haematologically as an acute, and likely reversible, thrombocytopenia.

Sub-Acute Toxicity (28-Day Study):

The third and most relevant objective for ethnomedicinal use was to assess the safety of the extract under conditions of repeated, daily administration. This 28-day study used lower, more practical doses (200, 400, and 800 mg/kg). The results from this sub-acute study (Table 3) were completely different from the acute toxicity study, highlighting a clear dose-dependent effect. The primary finding was that across all eight measured parameters (WBC, RBC, HGB, HCT, PLT, MPV, PDW, and PCT), there were no statistically significant differences ($p > 0.05$) between any of the extract-treated groups and the control group. This is arguably the study's strongest evidence supporting the extract's safety for traditional use. The stability of the RBC, HGB, and HCT counts over 28 days confirms that chronic use at these doses does not cause anaemia. It demonstrates that the extract does not have haemolytic properties and, importantly, does not appear to suppress the bone marrow's production of red blood cells (erythropoiesis). The stability of the total WBC count is equally important. It suggests the extract does not cause chronic inflammation (which would elevate WBCs) or immunosuppression (which would lower them) at these doses. The stable platelet (PLT) profile and its related indices (MPV, PDW, PCT) are striking. This finding proves that the acute stress response (thrombocytopenia) seen at 5000 mg/kg is a high-dose threshold effect that is completely absent at these lower, repeated doses. The extract does not disrupt platelet homeostasis during sub-acute administration. Although not statistically significant, the data in Table 3 points to a mild, non-significant increase in both WBC and PLT counts, especially at the 200 and 400 mg/kg doses. This suggests the extract may have mild immunostimulatory and thrombopoietic (platelet-stimulating) properties. This is a common and often beneficial characteristic of medicinal plants rich in phytochemicals like flavonoids and saponins, which are known immunomodulators (Agbai *et al.,* 2017).

The slight rise in platelets is likely a benign "reactive thrombocytosis," a response to cytokines that stimulate thrombopoietin (TPO) production (Thakkar and Marwaha, 2024). This biological activity, however, remains well within the normal, non-toxic physiological range for Wistar rats (Ozolua and Bafor, 2019).

The study findings successfully demonstrate the extract's dual-nature: At a massive, single acute dose (5000 mg/kg), the extract is not lethal but induces a significant, temporary physiological stress response, primarily characterized by acute thrombocytopenia (low platelets), likely due to splenic sequestration. Conversely, during repeated, long-term (28-day) administration at lower, practical doses (up to 800 mg/kg), the extract is shown to be exceptionally safe haematologically. It does not cause anaemia, disrupt immune cell counts, or affect platelet levels.

4.2 CONCLUSION

In conclusion, the methanol leaf extract of *T. mantaly* exhibited a high margin of safety and therefore, it is relatively safe at the tested doses. However, caution is seriously advised in its use at high doses, as it showed a suppressive effect either on the lifespan and/or on the production of blood cells, at the high acute dose of 5000 mg/kg.

4.3 Suggestions for Future Research

1. Chronic Toxicity:

Conduct a 90-day chronic toxicity study (OECD Guideline 408) to evaluate long-term, cumulative effects on bone marrow and immune function.

2. Genotoxicity:

Assess for carcinogenicity (OECD 451) and genotoxicity (e.g., Ames test, OECD 471/474) to check for mutagenic potential.

3. Expanded Models:

Replicate the study in male rats to check for sex-specific differences. Also, investigate reproductive and developmental toxicity (OECD 414/416).

4. Efficacy Studies:

Use the established low-toxicity profile to conduct efficacy studies in disease models, such as for malaria (antiplasmodial) or antimicrobial applications.

5. Human Trials:

Advance to Phase I human safety trials, prioritizing standardization of the extract and addressing potential drug interactions.

6. Nanoparticle Formulation:

Assess the toxicity of nanoparticle formulations derived from the extract for potential advanced applications, such as antitumor agents.

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