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ACUTE TOXICITY ASSESSMENT OF AQUEOUS LEAF EXTRACT IN MICE AND ITS IMPACT ON LIPID PROFILES AND HAEMATOLOGICAL PARAMETERS IN MALE WISTAR RATS: A STUDY of *Jatropha tanjorensis* J.L. ELLIS & SAROJA

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ABSTRACT

This study examines the acute toxicity of the aqueous extract of *Jatropha tanjorensis* leaf in mice and its effects on lipid profiles and haematological parameters in male Wistar rats. Acute toxicity assessment showed that graded doses of the extract up to 6000 mg/kg did not cause mortality. However, at 7000 mg/kg, a 33.33% mortality rate was observed, and 100% mortality occurred at 10,000 mg/kg. The extract caused a gradual decrease in total cholesterol (TC) at 400 mg/kg and 800 mg/kg. High-density lipoprotein cholesterol (HDL-C) increased in a dose-dependent manner. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) levels decreased. Atherogenic and coronary risk indices were significantly reduced ($p<0.05$) at 400 and 800 mg/kg, indicating improved cardiovascular health. Haematological analysis showed increased red blood cell (RBC) count and packed cell volume (PCV). Haemoglobin levels also increased significantly. Total white blood cell (TWBC) count increased with lower doses but decreased ($p<0.05$) at 800 mg/kg. Mean corpuscular volume (MCV) decreased, while mean corpuscular haemoglobin concentration (MCHC) increased. Differential WBC counts showed increased ($p<0.05$) neutrophils and variable lymphocyte percentages, with no detectable basophils. These results suggest that the extract has notable effects on lipid and haematological parameters, with significant toxicity at high doses, warranting further investigation into its safety and therapeutic potential.

Keywords: Aqueous leaf extract, Haematological parameters, *Jatropha tanjorensis*, Lipid profile, Wistar Rats

INTRODUCTION

Herbal medicines have long been valued for their natural origins and perceived low side effects, forming an essential part of local and regional healing traditions worldwide (WHO, 2019; Rizvi et al., 2022). According to the World Health Organization, these medicines are derived from plants with minimal industrial processing and are used for both the prevention and treatment of illnesses. Over centuries, they have been widely utilized in both developed and developing nations, progressing from simple forms like tinctures and teas to playing a key role in the development of modern drugs such as aspirin (from willow bark), morphine (from the opium poppy), and quinine (from cinchona bark) (Chaachouay& Zidane, 2024). Although the effectiveness of herbal remedies is well-established, concerns about their safety persist, leading to rigorous toxicity evaluations. While reports of serious adverse reactions are rare, the potential presence of harmful compounds such as alkaloids and anthraquinone glycosides necessitates thorough safety assessments (Zhang et al., 2015). This highlights the importance of ensuring the therapeutic benefits of medicinal plants outweigh any associated risks (Neergheen-Bhujun, 2013).

Jatropha tanjorensis J.L.Ellis&Sarojais a perennial shrub from the *Euphorbiaceae* family, widely recognized for its medicinal and nutritional benefits, especially in West African traditional medicine (Ellis & Saroja, 1961). The leaves are often utilized for their therapeutic properties, both in raw and processed forms. *Jatropha tanjorensis* is commonly known as "hospital-too-far" in some local dialects, signifying its perceived potency in treating various ailments (Ansari et al., 2020). The leaves are rich in bioactive compounds, such as Flavonoids, tannins, saponins, alkaloids, and phenolics (Ebhohon et al., 2024; Imohiosen, 2023; Ebenyi et al., 2021; Igbinaduwa et al., 2011). These compounds contribute to the plant's pharmacological effects, which include antioxidant (Ebhohon et al., 2024; Omoregie& Osagie, 2011), anti-inflammatory (Omoboyowa, 2021), antimicrobial (Ewa-Udu et al., 2022), and hypolipidemic properties (Oyewole&Akingbala, 2011).

Traditionally, the leaves are used as a blood tonic due to their ability to stimulate red blood cell production and enhance haemoglobin levels. This makes them useful in managing anaemia and other blood-related disorders (Ebenyi et al., 2021; Ansari et al., 2020; Ndem et al., 2019; Idu et al., 2014). The high content of flavonoids and other phenolic compounds in *Jatropha tanjorensis* leaves gives them significant antioxidant properties (Ebhohon et al., 2024; Omoregie & Osagie, 2011). These antioxidants help scavenge free radicals, protecting cells from oxidative damage and supporting overall health. Studies have shown that *Jatropha tanjorensis* leaves can positively affect lipid profiles by reducing total cholesterol, low-density lipoprotein (LDL), and triglycerides, while increasing high-density lipoprotein (HDL). This suggests a potential role in managing hyperlipidaemia and cardiovascular diseases (Oyewole&Akingbala, 2011). The leaves may boost the immune system by increasing white blood cell count, potentially enhancing the body's ability to fight infections. This is partly due to the presence of immune-modulating phytochemicals. There is evidence that *Jatropha tanjorensis* leaves may help regulate blood sugar levels, making them a candidate for managing diabetes and related metabolic disorders (Adebajo et al., 2004). The plant's leaves have shown promise in reducing inflammation and fighting microbial infections, making them useful in wound healing and the treatment of certain infections (Ewa-Udu et al., 2022; Omoboyowa, 2021)

The leaves of *Jatropha tanjorensis* are also consumed as a leafy vegetable, especially in parts of Nigeria, where they are

included in soups and sauces. They are a good source of essential nutrients, such as vitamins (especially vitamin C), minerals (such as iron, calcium, and potassium) and dietary fibre (Chigozie et al., 2018; Omobuwajo et al., 2011). This nutritional value supports their use as a food supplement in regions where micronutrient deficiencies are prevalent. While *Jatropha tanjorensis* is generally considered safe in traditional uses, some species within the *Jatropha* genus contain toxic compounds such as phorbol esters, which can be harmful if ingested in large quantities. It is essential to ensure proper preparation to avoid potential toxicity (Francis et al., 2021; Kumar et al., 2012).

Although *Jatropha tanjorensis* is widely used in traditional medicine and recognized for its pharmacological effects, including its impact on haematological parameters, further research is necessary to better understand how its aqueous leaf extract influences both haematological parameters and lipid profiles in animal models. The purpose of this study is to evaluate the acute toxicity and effects of *Jatropha tanjorensis* aqueous leaf extract on haematological parameters and lipid profiles in Wistar rats. This study aims to provide scientific evidence regarding the plant's potential therapeutic benefits for treating anaemia and improving lipid metabolism, while also evaluating its safety. The research seeks to clarify the balance between the plant's traditional medicinal uses and any potential risks associated with its consumption.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study were of analytical grade and procured from Sigma Chemical Company (St. Louis, MO, USA). Assay kits for cholesterol, and triacylglycerol were obtained from Randox (Antrim, U.K).

Collection of Plant Materials

Fresh *Jatropha tanjorensis* leaves were collected from the National Root Crops Research Institute (NRCRI) in Umudike, Abia State, Nigeria, and identified by Mr. Pipi Okey from the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike. The specimen was assigned voucher number MOUAU/PSB/18/103592 and deposited in the department's herbarium.

Preparation of Aqueous Extract of *Jatropha tanjorensis* Leaf

The leaves were thoroughly rinsed with distilled water to remove any debris and air-dried at room temperature until fully desiccated. The dried leaves were then ground into a fine powder using an electric blender. A measured quantity (188.54 g) of the powdered leaves was soaked in 2.5 L of distilled water for 24 hours in a sterile extraction jar. The resulting mixture was first filtered through muslin cloth and subsequently filtered again using Whatman No. 1 filter paper into a clean, calibrated flask. The filtrate was freeze-dried at a temperature of ≤ -40°C, yielding a dark-brown paste with a 50.85% yield. The paste was transferred into a sterile bottle and stored in a refrigerator at 4°C until it was needed for biochemical assays.

Experimental Animals

Twenty-four healthy male Wistar rats, each weighing approximately 160 ± 10 g, were obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. Prior to the experiment, the rats were acclimatized for at least one week to allow them to adjust to the laboratory environment and reduce stress related to transport and handling. The rats were housed in standard cages under controlled conditions, with the temperature set at 25 ± 2ºC and a 12-hour light/dark cycle. They were given free access to rat pellets as their standard diet and clean drinking water throughout the experiment. All animal care and handling procedures adhered strictly to the National Institute of Health's guidelines for the care and use of laboratory animals, as well as the approved protocols of the Animal Care and Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State with approval number CVET/029/23. Every effort was made to minimize the number of animals used and to prevent any potential discomfort or distress during the study.

Acute Toxicity Study

Acute toxicity evaluation was performed using a slightly modified version of the method described by Lorke (1983) to determine the LD_{50}. In Phase 1, nine male Wistar rats, after a one-week acclimatization period, were divided into three groups (n = 3) and administered 10, 100, and 1000 mg/kg body weight of *Jatropha tanjorensis* aqueous leaf extract. The rats were observed for 24 hours to monitor any behavioural changes or mortality. In Phases 2 and 3, eighteen additional male Wistar rats were divided into three groups (n = 3) and administered doses of 1600, 2900, and 5000 mg/kg of the extract for Phase 2, and 6000, 7000, and 10,000 mg/kg of the extract for Phase 3. The rats were again monitored for 24 hours for signs of toxicity or death (Tables 1, 2, and 3). The lethal dose (LD_{50}) was calculated using the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$, where D_0 is the highest dose that did not result in death, and D_{100} is the lowest dose that caused mortality.

Sub-Acute Toxicity Study

The rats were randomly divided into four groups (n = 6 per group):

Group 1 (Control): Rats received 0.5 mL of distilled water orally once a day for 28 days.

Group 2 (200 mg/kg): Rats received 200 mg/kg body weight of the aqueous extract *Jatropha tanjorensis* leaf using an oral gavage daily for 28 days.

Group 3 (400 mg/kg): Rats received 400 mg/kg body weight of the aqueous extract *Jatropha tanjorensis* leaf using an oral gavage daily for 28 days.

Group 4 (800 mg/kg): Rats received 800 mg/kg body weight of the aqueous extract *Jatropha tanjorensis* leaf using an oral gavage daily for 28 days.

The animals were monitored for signs of toxicity and mortality throughout the duration of the study (Pillai et al., 2011).

Biochemical Assay

Blood Sample Collection

At the end of the experiment, all the rats were anaesthetized with an intraperitoneal injection of 25% urethane and sacrificed immediately. Blood samples were drawn using a 5 ml syringe via cardiac puncture (Arunachalam and Sasidharan, 2021) into plain sample tubes, and centrifuged at 4000 rpm for 10 minutes to obtain serum which was used for biochemical assays in this study.

Lipid Profile Tests

Total cholesterol (TC), triacylglycerol (TAG), and high-density lipoprotein (HDL) were estimated using Randox kits. Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) values were calculated by using the Friedewald (Frieldwald et al., 1972) formula.

LDL-cholesterol = Total cholesterol – HDL- cholesterol – (concentration of Triacylglycerol)/5
VLDL-cholesterol = (Concentration of Triacylglycerol) /5
Atherogenic index (AI) and coronary risk index (CRI) were calculated as: LDL-c (mg/dl)/HDL-c (mg/dl) (Abbott et al.,1988) and TC (mg/dl)/HDL-c (mg/dl) (Alladi et al.,1989)

Measurement of Haematological Parameters
Blood samples collected via cardiac puncture were dispensed into EDTA-capped sample tubes with the aid of a 5 ml syringe. The blood samples were then used for evaluating various haematological parameters such as red blood cell (RBC) count, total white blood cell (WBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC): (Laposata and McCaffrey, 2022). The blood samples were analysed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK).

Statistical Analysis
Data are presented as mean ± SEM. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare differences between means. Both ANOVA and Tukey's post-hoc tests were performed using GraphPad Prism, version 7. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity Study of Aqueous Extract of *Jatropha tanjorensis* Leaf in Mice
Graded doses of the extract, up to 6000 mg/kg, did not result in any mortality, as the treated mice remained active and physically stable throughout the 24-hour observation period. However, a 33.33% mortality rate was observed in the third phase, where mice received 7000 mg/kg, while all mice in the group administered 10,000 mg/kg died, resulting in 100% mortality. Using Lorke's formula, the LD_{50} value of the aqueous extract was calculated to be 7745.97 mg/kg body weight. The detailed results are presented in Tables 1, 2, and 3 below.

Table 1: Phase 1: Acute toxicity (LD_{50}) evaluation of the extract in Mice

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	100	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	1000	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 2: Phase 2: Acute toxicity (LD_{50}) evaluation of the extract in Mice

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	1600	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	2900	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
3	5000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within one hour of administration.

Table 3: Phase 3: Acute toxicity (LD_{50}) evaluation of the extract in Mice

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	6000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity 24 hours of administration.
2	7000	1/3	33.33	33.33% mortality was observed. Surviving animals were weak, depressed and calm. They also did not completely regain physical activity within 24 hours of administration.
3	10000	3/3	100.00	Rats were initially calm and depressed and 100% mortality was observed by the end of 24 hours of administration.

$LD_{50} = (D_0 \times D_{100})^{1/2}$
Where:
D_0: Highest dose that gave no mortality.
D_{100}: Lowest dose that produced mortality
$LD_{50} = (6000 \times 10000)^{1/2}$
LD_{50} = 7745.97 mg/kg body weight

Table 4: Effect of Varying Doses of Aqueous Extract of *Jatropha tanjorensis* Leaf on the Lipid Profile in Serum of Male Wistar Rats

Treatment groups	Total Cholesterol (mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Control	94.22±2.80^{b}	56.12±1.02^{a}	67.51±2.45	24.61±3.97^{b}	13.50±0.49^{c}
Jatropha tanjorensis (200 mg/kg)	89.27±1.88^{ab}	59.53±0.62^{b}	60.93±2.24^{b}	17.56±1.66^{ab}	12.19±0.45^{b}
Jatropha tanjorensis (400 mg/kg)	81.81±3.71^{a}	61.30±0.66^{bc}	54.49±1.74^{a}	9.61±3.08^{a}	10.90±0.35^{a}
Jatropha tanjorensis (800 mg/kg)	83.02±1.27^{a}	62.66±0.33^{c}	58.20±0.27^{ab}	8.71±1.47^{a}	11.64±0.05^{ab}

Data are expressed as Mean ± SEM. Values sharing the same lowercase letters indicate no significant difference (P > 0.05), while those with different lowercase letters are significantly different (P < 0.05).

In Table 4, the lipid profile analysis of male Wistar rats treated with varying doses of aqueous extract of *Jatropha tanjorensis* shows significant effects on serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TAG), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C). The total cholesterol levels decrease progressively with higher doses of the extract, with a significant ($p<0.05$) reduction at 400 mg/kg and 800 mg/kg compared to the control. HDL-C, often referred to as "good cholesterol," showed a dose-dependent increase across the treatment groups. The highest dose (800 mg/kg) results in the most significant ($p<0.05$) elevation in HDL-C levels. Triglyceride levels show a significant ($p<0.05$) reduction with the 400 mg/kg dose, although the 800 mg/kg dose causes a slight increase compared to 400 mg/kg. LDL-C, or "bad cholesterol," shows a significant ($p<0.05$) decrease in the treated groups, especially at 400 mg/kg and 800 mg/kg. VLDL-C levels also show a decline ($p<0.05$), especially at 400 mg/kg,

Table 5: Effect of Varying Doses of Aqueous Extract of Jatropha *tanjorensis* Leaf on Atherogenic Index and Coronary Risk Index of Male Wistar Rats

Treatment groups	Atherogenic Index	Coronary Risk Index
Control	0.4428 ±0.07^{a}	1.683±0.07^{a}
Jatropha tanjorensis (200 mg/kg)	0.2948 ± 0.02^{a}	1.500 ±0.02^{a}
Jatropha tanjorensis (400 mg/kg)	0.1555 ± 0.04^{b}	1.333±0.04^{b}
Jatropha tanjorensis (800 mg/kg)	0.1395 ± 0.02 ^{b}	1.325±0.02^{b}

Data are expressed as Mean ± SEM. Values sharing the same lowercase letters indicate no significant difference (P > 0.05), while those with different lowercase letters are significantly different (P < 0.05).

The data in Table 6 show the effect of varying doses of *Jatropha tanjorensis* leaf aqueous extract on two cardiovascular risk markers, the Atherogenic Index (AI) and Coronary Risk Index (CRI), in male Wistar rats. At a dose of 200 mg/kg, AI and CRI were both reduced ($p<0.05$) compared to the control, indicating a mild decrease in cardiovascular risk. A dose of 400 mg/kg resulted in a significant ($p<0.05$) reduction in AI and CRI, reflecting a stronger protective effect. At 800 mg/kg, AI and CRI remained significantly ($p<0.05$) lower than in the control, with minimal difference from the 400 mg/kg dose, suggesting that increasing the dose beyond 400 mg/kg may not yield additional cardiovascular benefits. The Atherogenic Index and Coronary Risk Index decrease progressively with increasing doses of the extract, with a significant ($p<0.05$) reduction observed at 400 mg/kg and 800 mg/kg compared to the control. As a result, both the 400 mg/kg and 800 mg/kg doses demonstrate significant improvements in AI and CRI when compared to the control and the 200 mg/kg group.

The results of the study examining the effect of varying doses of the aqueous extract of *Jatropha tanjorensis* leaf on haematological parameters in Male Wistar rats reveal several key observations. The RBC count increased ($p<0.05$) with the extract treatment, reaching a peak at 400 mg/kg, and remained elevated at 200 mg/kg and 800 mg/kg compared to the control. PCV values were higher ($p<0.05$) in all extract-treated groups compared to the control, with the highest value observed in the 400 mg/kg group. Haemoglobin levels also increased ($p<0.05$) with extract treatment, peaking at 400 mg/kg. TWBC increased ($p<0.05$) with 200 and 400 mg/kg doses but decreased at 800 mg/kg. The platelet count remained relatively stable across different doses, indicating that the extract does not significantly ($p<0.05$) affect platelet production or destruction. MCV values decreased ($p<0.05$) with the extract treatment but remained within a normal range, suggesting no significant ($p<0.05$) alteration in red blood cell size. MCH values were similar across the groups, indicating no significant ($p<0.05$) change in the average amount of haemoglobin per red blood cell. MCHC increased ($p<0.05$) in the 200 mg/kg group and remained higher compared to the control at higher doses.

Table 6: Effect of Varying Doses of Aqueous Extract of *Jatropha tanjorensis* Leaf on Haematological Parameters in Male Wistar Rats

Treatment groups	RBC ($x10^6/mm^3$)	PCV (%)	Hb (g/dl)	TWBC ($x10^3/mm^3$)	PLT ($x10^3/mm^3$)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	6.82 ± 0.12^{a}	44.00 ± 0.41^{a}	15.55 ± 0.17^{a}	8.36 ± 0.27^{a}	239.25 ± 2.17^{a}	64.60 ± 0.65^{b}	22.83 ± 0.22^{a}	35.34 ± 0.12^{a}
Jatropha tanjorensis (200 mg/kg)	7.36 ± 0.09^{b}	46.25 ± 0.63^{b}	16.93 ± 0.24^{b}	9.05 ± 0.20^{ab}	241.75 ± 2.43^{a}	62.86 ± 0.34^{a}	23.01 ± 0.13^{a}	36.60 ± 0.04^{b}
Jatropha tanjorensis (400 mg/kg)	7.56 ± 0.10^{b}	47.25 ± 0.48^{b}	17.05 ± 0.16^{b}	9.26 ± 0.24^{b}	240.00 ± 3.42^{a}	62.56 ± 0.65^{a}	22.58 ± 0.22^{a}	36.09 ± 0.39^{b}
Jatropha tanjorensis (800 mg/kg)	7.41 ± 0.12^{b}	46.50 ± 0.65^{b}	16.85 ± 0.25^{b}	8.78 ± 0.12^{ab}	239.00 ± 3.76^{a}	62.77 ± 0.28^{a}	22.74 ± 0.11^{a}	36.24 ± 0.11^{b}

Data are expressed as Mean ± SEM. Values sharing the same lowercase letters indicate no significant difference ($P > 0.05$), while those with different lowercase letters are significantly different ($P < 0.05$).

Table 7: Effect of Varying Doses of Aqueous Extract of *Jatropha tanjorensis* Leaf on Differential WBC in Male Wistar Rats

Treatments groups	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Control	39.25 ± 0.75^{a}	54.00 ± 0.82^{b}	4.50 ± 0.29^{a}	2.25 ± 0.25^{a}	0.00 ± 0.00^{a}
Jatropha tanjorensis (200 mg/kg)	38.75 ± 1.70^{a}	54.25 ± 1.70^{ab}	4.50 ± 0.29^{a}	2.50 ± 0.29^{a}	0.00 ± 0.00^{a}
Jatropha tanjorensis (400 mg/kg)	43.00 ± 1.29^{a}	49.50 ± 1.32^{a}	4.75 ± 0.25^{a}	2.75 ± 0.25^{a}	0.00 ± 0.00^{a}
Jatropha tanjorensis (800 mg/kg)	42.75 ± 1.31^{a}	51.00 ± 1.58^{ab}	4.00 ± 0.41^{a}	2.25 ± 0.25^{a}	0.00 ± 0.00^{a}

Data are expressed as Mean ± SEM. Values sharing the same lowercase letters indicate no significant difference ($P > 0.05$), while those with different lowercase letters are significantly different ($P < 0.05$).

The results detailing the effect of varying doses of aqueous extract of *Jatropha tanjorensis* leaf on differential white blood cell (WBC) counts in Male Wistar rats provide insights into the extract's impact on different types of white blood cells. The percentage of neutrophils showed a significant ($p<0.05$) increase at the 400 mg/kg and 800 mg/kg doses compared to the control However, this effect appears to plateau or stabilize at higher doses. Lymphocyte percentages decreased slightly ($p<0.05$) at the 400 mg/kg dose but increased again at 800 mg/kg. The decrease ($p<0.05$) at 400 mg/kg might suggest a temporary shift in immune response or a modulation effect of the extract, while the increase at 800 mg/kg could indicate a compensatory response or restoration of lymphocyte levels. The percentage of monocytes remained relatively stable across most groups, with a slight increase ($p<0.05$) at 400 mg/kg and a slight decrease ($p<0.05$) at 800 mg/kg. Eosinophil percentages increased slightly ($p<0.05$) at 200 mg/kg and 400 mg/kg doses but returned to control levels at 800 mg/kg. Basophils were undetectable in all treatment groups. The absence of basophils across all groups suggests that the extract does not affect basophil levels or that the baseline levels were already low.

Discussion

This study provides key insights into the safety and toxicity of the aqueous extract of *Jatropha tanjorensis*. Oral administration of the extract at doses up to 6000 mg/kg demonstrated no mortality and no acute toxic effects, as treated mice remained active and stable throughout the 24-hour observation period. However, higher doses, such as 7000 mg/kg, resulted in noticeable toxicity.

This indicates that the extract begins to exhibit toxic effects at this dose. The increased mortality at this level highlights the dose-dependent nature of the extract's toxicity. The complete mortality (100%) observed at the highest dose of 10,000 mg/kg indicates a significant level of toxicity. This finding emphasizes that the extract has a narrow safety margin at high doses, suggesting that careful dose management is crucial to avoid severe adverse effects. The LD_{50} value, calculated using Lorke's formula, is 7745.97 mg/kg body weight. This value provides an estimate of the dose at which 50% of the test population is expected to die. The LD_{50} is a critical parameter in assessing the acute toxicity of the extract. Given that the LD_{50} value is quite high compared to the doses that resulted in mortality, it implies that the extract has a relatively high threshold for acute toxicity, but it is not without risk, particularly at very high doses. This result aligns with the findings of Ebenyi et al. (2021), who reported no behavioural changes or mortality within the first 24 hours or during a 14-day observation period following the oral administration of 2,000 and 5,000 mg/kg of aqueous *Jatropha tanjorensis* leaf extract. Similarly, Igbinaduwa et al. (2011) found no mortality, behavioural changes, or signs of toxicity in mice and rats treated with methanol extract of *Jatropha tanjorensis* leaf at doses up to 8,000 mg/kg body weight. In summary, the study demonstrates that *Jatropha tanjorensis* extract is relatively safe at lower to moderate doses but becomes increasingly toxic at higher doses. The LD_{50} value provides a quantitative measure of the extract's toxicity, which is essential for determining safe dosage ranges and guiding further research and application.

In this study, the results indicate that the aqueous extract of *Jatropha tanjorensis* positively influences lipid metabolism. Specifically, it demonstrates a dose-dependent reduction in total cholesterol, LDL-C, and VLDL-C, alongside a dose-dependent increase in HDL-C levels. These findings suggest that the extract has potential benefits for preventing hypercholesterolemia and associated cardiovascular diseases. The observed reduction in LDL-C is particularly noteworthy, as lower LDL-C levels are crucial for mitigating the risk of atherosclerosis and other cardiovascular conditions. This indicates that *Jatropha tanjorensis* extract may offer protective effects against such diseases by effectively lowering LDL-C. Additionally, the decrease in serum VLDL-C levels suggests that the extract could be beneficial in diminishing circulating VLDL-C, which plays a significant role in triglyceride transport and metabolism. This reduction is advantageous as high VLDL-C levels are linked to an increased risk of cardiovascular disorders. Conversely, the extract's capacity to increase HDL-C is promising, as higher HDL-C levels are associated with improved cardiovascular health. Elevated HDL-C is known for its protective role against heart disease. The lower triglyceride levels observed also imply that *Jatropha tanjorensis* might contribute to a reduced risk of metabolic disorders, highlighting its potential as a lipid-lowering agent, particularly at moderate doses. These effects were most pronounced at moderate to higher doses (400 mg/kg and 800 mg/kg), indicating that the plant may be effective in managing hyperlipidaemia and promoting cardiovascular health. My findings differ from those of Oyewole et al. (2011). According to their report, there were significant reductions in serum total cholesterol and LDL cholesterol, while triglyceride and HDL cholesterol levels remained unaffected. In contrast, this study demonstrated significant decreases in total cholesterol (TC), triglycerides (TG), LDL-C, and VLDL-C, along with a notable increase in HDL-C in the serum of the treated animals. However, the results of this study were consistent with those of Srivastava et al. (2023), where both aqueous and ethanol extracts of *Jatropha tanjorensis* leaf significantly reduced TC, TG, and LDL-C levels, while significantly increasing HDL-C in rats with high-fat diet-induced obesity. Further studies on the mechanism of action and long-term effects would be beneficial to establish its therapeutic potential fully. Atherogenic and coronary artery indices are reliable indicators and independent assessors of cholesterol uptake, metabolism, and excretion, with elevated reference values suggesting a higher risk of developing coronary artery disease (Hartog et al., 1993; Kanekt et al., 1996). These markers play a crucial role in evaluating cardiovascular health, as lower values reflect a decreased risk of atherosclerosis and coronary artery disease. The reference values for values for atherogenic index and coronary artery index in human should not be greater than 4 and 2.5, respectively (Murray and Pizzorno 1998). The reduction. in atherogenic and coronary indices by the extract strongly suggests its cardioprotective potential. In inference, the extract seems to have a multifaceted impact on lipid profiles by influencing various aspects of lipid metabolism. It reduces harmful fats while increasing beneficial ones, suggesting its potential as a natural remedy for maintaining a healthier lipid balance, which is vital for cardiovascular disease prevention. Additional studies could further establish its role in lipid management and cardiovascular health.

The aqueous extract of *Jatropha tanjorensis* demonstrates a notable impact on various haematological parameters, with significant effects observed particularly at the 400 mg/kg dosage. The increase in red blood cell (RBC) count, packed cell volume (PCV), and haemoglobin (Hb) levels at this dose suggests an enhancement in the blood's oxygen-carrying capacity and overall hematologic health. The elevated RBC count observed indicates that the extract may stimulate

erythropoiesis, which could either enhance the production of red blood cells or reduce their destruction. This is further supported by the increase in PCV, reflecting a greater proportion of blood volume occupied by red blood cells. The higher Hb levels corroborate the increased RBC count and PCV, suggesting improved oxygen delivery throughout the body. These findings align with those reported by Ebenyi et al. (2021) and Ndem et al. (2019), who investigated the effects of aqueous leaf extract of *Jatropha tanjorensis* on haematological parameters in mice infected with *Plasmodium berghei*. Additionally, the increase in total white blood cell count (TWBC) at lower doses points to a potential enhancement in immune function. However, this effect appears to diminish at higher doses, suggesting that while the extract may boost immune responses at moderate levels, excessive doses might reduce this benefit or lead to other adverse effects. Platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) remained stable or within normal ranges across all treatment groups. This stability indicates that the extract does not negatively affect platelet function or cause significant alterations in red blood cell morphology and haemoglobin concentration. My findings differ from those of Ndem et al. (2019). In her study, there was a significant decrease in TWBC and a significant increase in platelet count, whereas my research showed significant increases in both TWBC and platelet count. On the other hand, my results are slightly aligned with the study by Ebe and Chukwuebuka (2019), which investigated the effects of ethanol root extract of *Jatropha tanjorensis* on haematological indices in male albino Wistar rats. Their study found that MCV, MCH, and MCHC values at lower and moderate doses did not differ significantly from the control, whereas the highest dose showed significantly higher values. In contrast, my research revealed that MCV, MCH, and MCHC values remained within normal ranges across all treatment groups. In brief, the extract exhibits potential for improving haematological parameters, especially at moderate doses. Its effects on RBC production, PCV, and Hb suggest beneficial impacts on blood health. However, the variations in TWBC across doses highlight the need for further research to fully understand its implications for immune function and to establish safe and effective dosing strategies.

The aqueous extract of *Jatropha tanjorensis* demonstrates a modulating effect on various aspects of the immune system in Male Wistar rats, as evidenced by changes in differential white blood cell counts. The most notable effect is observed in neutrophil counts, which increase significantly at higher doses. Neutrophils, crucial for the innate immune response, play a key role in defending against bacterial infections and responding to inflammation. The observed increase suggests that the extract may enhance neutrophil function, potentially improving the body's ability to combat infections and manage inflammatory responses. In contrast, the variations in lymphocyte percentages indicate a more complex interaction with adaptive immunity. Lymphocytes are essential for the adaptive immune response, including the management of viral infections and immune surveillance. The slight decrease in lymphocyte counts at the 400 mg/kg dose, followed by an increase at 800 mg/kg, could reflect a dose-dependent modulation of the adaptive immune response, suggesting that the extract may influence lymphocyte activity and distribution in a non-linear manner. The minimal changes in monocyte levels across treatment groups, coupled with the return to baseline eosinophil levels at higher doses, suggest that the extract's effects on these cell types are relatively subtle and dose-dependent. Monocytes, which are involved in phagocytosis and inflammatory response regulation, show only slight variations, indicating that the extract may have a more pronounced effect on other white blood cell types rather than directly influencing monocyte counts. The eosinophil counts increased slightly at lower doses but normalized at higher doses, hinting at a potential stimulatory effect at lower doses and a regulatory mechanism at higher doses. Interestingly, basophils were undetectable across all treatment groups. Basophils, which are involved in allergic reactions and inflammation, were not affected by the extract, indicating that the extract does not significantly influence this cell type. Overall, the extract of appears to differentially impact white blood cell subtypes, with significant effects on neutrophils and lymphocytes while having less pronounced effects on monocytes and eosinophils. The absence of any effect on basophils further underscores the specificity of the extract's influence on immune cell populations. My findings differ from those of Ndem et al. (2019), who reported significant decreases in differential WBC counts. In contrast, my study found notable effects on neutrophils and lymphocytes, with less pronounced changes in monocytes and eosinophils. Additionally, no effects were observed on basophils. Future research should focus on elucidating the underlying mechanisms of these effects and exploring the broader implications for immune function and overall health.

CONCLUSION

In summary, the aqueous extract of *Jatropha tanjorensis* leaf demonstrates a favourable safety profile at lower doses but exhibits increased toxicity at higher concentrations, with an LD_{50} of 7745.97 mg/kg. The extract also shows cardioprotective and lipid-lowering potential, as indicated by dose-dependent reductions in total cholesterol, LDL-C, VLDL-C, and triglycerides, along with elevated HDL-C levels. This suggests its potential role in managing hyperlipidaemia and promoting cardiovascular health, further supported by its dose-dependent reduction of the atherogenic and coronary risk indices. Moderate doses of the extract positively influence haematological parameters, increasing RBC count, PCV, Hb levels, and potentially enhancing immune function by boosting TWBC and neutrophil counts. While these findings highlight the extract's potential for improving cardiovascular and haematological health, further studies are necessary to explore its mechanisms and establish safe dosing for clinical use.

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