

HEMATOLOGICAL AND IMMUNOLOGICAL EFFECTS OF *KALANCHOE PINNATA* EXTRACTS AGAINST CARBON TETRACHLORIDE (CCl₄) INDUCED TOXICITY IN MALE WISTAR RATS

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Article Info: Received: 06 July 2026, Revised: 26 August 2026, Accepted: 16 September 2026

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DOI: <https://doi.org/10.5281/zenodo.23065685>



Citation:

Iboyi Nathaniel Onuche^{*1}, Ebugosi, Richard S.², Achara Ngozichukwuka Immaculata², Ogalagu Romanus² (2026). Hematological And Immunological Effects Of *Kalanchoe Pinnata* Extracts Against Carbon Tetrachloride (Ccl₄) Induced Toxicity In Male Wistar Rats. International Journal of Clinical and Pharmaceutical Innovations, 1(7), 49-55.

ABSTRACT

The ethanolic extract of *Kalanchoe pinnata* (*Bryophyllum pinnatum*) was assessed for haematological and immunological effects in male Wistar rats exposed to CCl₄-induced toxicity. Forty-two rats were randomly allocated into seven groups: a normal control group, a CCl₄-induced negative control group, three treatment groups (200, 400, and 600 mg/kg extract administered post-induction), a silymarin-treated positive control group, and a prophylactic group pre-treated with extract. The plant extract was administered orally for 30 days, while CCl₄ was injected intraperitoneally. An automated haematology analyser was utilised to assess the liver-to-body weight ratio, white blood cell indices, and various other blood parameters. The findings demonstrated that CCl₄ exposure resulted in hepatotoxicity, as indicated by an increased liver-to-body weight ratio and significant decreases in packed cell volume, haemoglobin concentration, and red blood cell count, indicating anaemia. Leukocytosis, neutrophilia, thrombocytosis, and modified leukocyte differentials indicated inflammatory and immunological impairment. The extract of *Kalanchoe pinnata* enhanced these alterations in a dose-dependent manner. Elevated doses (400 and 600 mg/kg) restored normal blood levels, reduced liver size, and normalised white blood cell markers, akin to silymarin. The extract markedly increased mean corpuscular indices, indicating improved erythropoiesis and defence against oxidative haemolysis. The plant contains numerous phytochemicals, such as flavonoids and phenolic compounds, which are beneficial due to their antioxidant, anti-inflammatory properties, and membrane-stabilizing effects. *Kalanchoe pinnata* exhibits hematoprotective and immunomodulatory effects against CCl₄-induced toxicity, supporting its traditional uses and suggesting its potential as a natural treatment for toxin-induced haematological and immunological problems.

KEYWORDS: *Bryophyllum pinnatum*, Carbon tetrachloride (CCl₄), Hepatotoxicity, Haematological parameters, Immunomodulatory effects.

INTRODUCTION

Many developing countries, including Nigeria, continue to depend largely on herbal remedies for the management of various diseases despite the availability of conventional medicine. This reliance is attributed to the high cost, limited accessibility, and potential adverse effects of orthodox drugs used in the treatment of liver disorders. Consequently, there has been increasing interest in identifying affordable, effective, and safer therapeutic alternatives from medicinal plants, particularly those with antioxidant properties (Sakshi et al., 2024).

Kalanchoe pinnata (also known as ***Bryophyllum pinnatum***) is a perennial herb that can grow to be 3 to 5 meters tall. Its leaves are smooth and grow opposite each other. The plant has a lot of bioactive chemicals that give it a wide range of pharmacological effects. Traditionally, it is used to treat coughs, bug bites, skin infections, psychological illnesses, burns, rheumatoid arthritis, and stomach pain (Sakshi et al., 2024). It is also used to treat a number of conditions, such as conjunctivitis, oedema, piles, eczema, constipation, epilepsy, asthma, fever, and menstrual problems (Adegboyega et al., 2002). It is known for its anti-inflammatory, pain-relieving, wound-healing, and blood-stopping properties (Gupta et al., 2016). Additionally, slightly warmed leaves are employed as a tocolytic drug to avert premature labour and to treat superficial skin infections, as well as to facilitate placental ejection in certain regions of Southeast Nigeria (Oufir et al., 2015). Other portions of the plant, such as the roots, have laxative, diuretic, and antipsychotic properties. People also use crushed leaf paste on their skin to cure boils and abscesses (Elufioye et al., 2022). In Germany, anthroposophical medicine prescribes preparations of *Kalanchoe pinnata* for tocolysis and behavioural disorders.

Environmental pollutants, especially carbon tetrachloride (CCl₄), have played a big role in the worldwide rise in liver illnesses. People can be exposed to CCl₄ by breathing it in, swallowing it, or touching it with their skin in places that are contaminated. CCl₄ is a colourless, volatile, and non-flammable chemical. When cytochrome P450 enzymes break it down, it becomes quite poisonous and makes reactive free radicals like trichloromethyl (CCl₃•). These radicals start lipid peroxidation, which damages cells, causes inflammation, and makes many organs not work properly (Saleh, 2016). CCl₄ is known to be toxic to the liver. It makes trichloromethyl and trichloromethyl peroxy radicals in liver cells, which hurts the liver's structure and function. Research has demonstrated that CCl₄-induced toxicity impacts haematological parameters (Ribeiro et al., 2019).

The liver is an important part of metabolism and detoxification because it has complex enzyme systems that control blood sugar levels and keep red blood cells healthy. However, being around harmful substances that cause oxidative stress can make the body make too many

reactive oxygen species, which can seriously damage the liver, lead to bad clinical results, and cause high death rates because there aren't many good treatments available. Researchers often utilise CCl₄ in experiments to look at hepatotoxicity because it produces lipid peroxidation and damage to the liver and other organs, such as the kidneys, heart, brain, and testes. Oxidative stress is especially bad for the liver because it constantly generates toxic metabolites and inflammatory mediators. CCl₄ poisoning has been linked to hepatomegaly, anaemia, leukocytosis, and immunological dysfunction due to oxidative damage and lipid peroxidation (Abdel-Moneim et al., 2020).

Medicinal plants abundant in antioxidants have garnered significant interest in recent years due to their preventive properties against oxidative damage. *Kalanchoe pinnata* is one such plant that is rich in flavonoids, phenolic compounds, and other bioactive substances that have antioxidant, anti-inflammatory, and hematoprotective effects (Akinpelu et al., 2014). The growing problem of antibiotic resistance has made people even more interested in natural products as possible treatments. Nonetheless, despite their extensive traditional application, only a select few of these plants have undergone scientific validation for their antibacterial properties (Iqbal et al., 2006). Medicinal plants remain a significant source of bioactive compounds that can be refined to enhance therapeutic efficacy while reducing toxicity (Mostofa et al., 2017). Plants are very important to health care since they provide safe and efficient treatments for many diseases. *Bryophyllum pinnatum*, sometimes referred to as *Kalanchoe pinnata*, the "Life Plant," and the "Miracle Plant," is extensively utilised in traditional medicine in Nigeria (Nwadinigwe, 2011). This study sought to assess the haematological and immunological impacts of ethanolic extract of *Kalanchoe pinnata* in rats subjected to CCl₄-induced toxicity, concentrating on liver-to-body weight ratio, complete blood count parameters, and white blood cell indices.

MATERIAL AND METHODS

Collection of Plant Sample

Kalanchoe pinnata leaves were collected from Ibusa in Oshimili North Local Government Area of Delta State, Nigeria. The plant was authenticated at the Botany Department of Nnamdi Azikiwe University, Awka, where a voucher specimen (Herbarium No: NAUTH-172A) was deposited. The freshly harvested leaves were washed thoroughly with clean running water, air-dried under shade at room temperature, and then ground into a coarse powder. The powdered sample was stored in a clean, dry, and sterile container for further extraction.

Sample Preparation

Extraction

Five hundred grams of the powdered leaves were soaked in 1.5 L of ethanol for 48 hours. The mixture was first filtered using a mesh cloth and then with Whatman No. 1

filter paper. The filtrate was concentrated using a water bath at 100 °C to evaporate the solvent, and the resulting extract was weighed and stored in a refrigerator for subsequent analysis.

Experimental Animals

A total of 42 adult male Wistar rats (weighing 140–210 ± 20 g) were obtained from the Animal House of the Federal University of Technology, Owerri, Nigeria. The animals were housed in well-ventilated cages with sawdust bedding under a 12-hour light/dark cycle. They were acclimatized for 10 days at the Animal House of Admiralty University of Nigeria, Ibusa, and maintained under controlled environmental conditions (25 ± 2 °C and 40–70% humidity). The rats had free access to standard feed and water throughout the study. Ethical approval was obtained, and all procedures complied with established animal care guidelines.

Experimental design

The 42 rats were randomly grouped after acclimatization into 7 groups containing 6 rats each for the evaluation of chemoprotective and ameliorative effects of ethanolic extract of *Kalanchoe pinnata* in CCl₄ induced hepatocellular injuries in albino rats.

Group A (Control): Fed with normal chop and water daily for the period of the experiment

Group B: served as the negative control, It was induced with 1 mL/kg body weight of carbon tetrachloride (CCl₄) intraperitoneally without any treatment.

Group C: It was administered 200 mg/kg body weight of ethanolic extract of *Kalanchoe pinnata* daily for 30 days after induced with CCl₄

Group D: It was administered 400 mg/kg body weight of ethanolic extract of *Kalanchoe pinnata* for 30 days after induced with CCl₄

Group E: It was administered 600 mg/kg body weight of ethanolic extract of *Kalanchoe pinnata* daily for 30 days after induced with CCl₄

Group F: served as the positive control, it was induced with 1 mg/kg/day b.wt. CCl₄ intraperitoneally and treatment with a standard drug for 30 days.

Group G: was administered 400 mg/kg/day b. wt. orally of extract of *Kalanchoe pinnata* respectively for 30 days before induced with CCl₄ intraperitoneally.

In the experimental procedure, carbon tetrachloride (CCl₄) was administered to the animals via intraperitoneal injection, while the plant extract was given orally over a period of 30 days. At the end of the treatment duration, the animals were fasted overnight for 12 hours, after which they were sacrificed and their final body weights recorded. Blood samples were subsequently collected.

Full Blood Count (FBC) Using Automated Hematology Analyzer (Sysmex XP-300)

Sample Collection

Blood samples were collected via venipuncture into EDTA anticoagulant bottles and gently mixed to prevent

clotting. Samples were analyzed within 4 hours of collection.

Hemoglobin: Hemoglobin concentration was determined photometrically using the cyanmethemoglobin method, based on absorbance measurement at a specific wavelength, as described by Bain *et al.*, (2022).

WBC differential: Differential white blood cell counts were determined using automated flow cytometry with laser light scatter technology, which classifies leukocyte populations based on cell size, granularity, and nucleic acid content (Briggs *et al.*, 2012).

RBC indices: Red blood cell indices, including packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were automatically calculated by the hematology analyzer using impedance and optical measurements (Hoffbrand *et al.*, 2023).

Data Analysis

Experimental data are presented as Means ± Standard Error Mean (SEM). Statistical analysis was implemented using the computer software SPSS 23.0 version statistical package program (SPSS, Chicago, IL). One-way analysis of variance was used to compare variables among the different groups. Level of significance (Post hoc 39 comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at p<0.05.

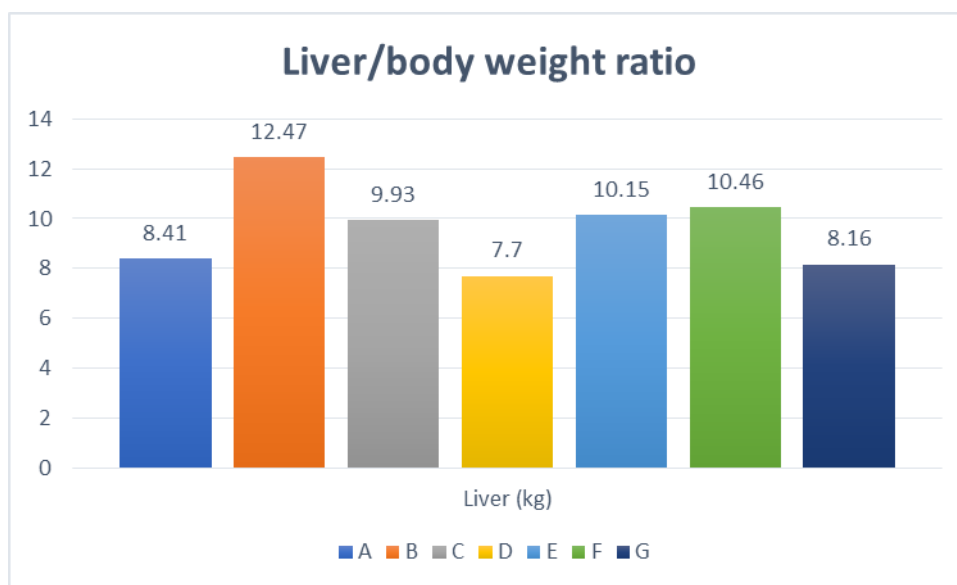
RESULTS

Impact/Effects of Ethanolic Extract of *Kalanchoe pinnata* on Liver–Body Weight Ratio

The significant elevation in the liver-to-body weight ratio in the negative control group (Group B), recorded at 12.47 ± 0.30 compared to the control group at 8.41 ± 0.01 as shown in Table 12, indicates hepatomegaly, a hallmark of toxic liver injury resulting from inflammatory cell infiltration, lipid accumulation, vascular congestion, and hepatocellular oedema. The liver body weight varies from 7.70 ± 0.10 to 12.47 ± 0.30, with some values near and others exceeding the normal range represented by the control group. There was a substantial change in the weight of the liver across all groups when compared to the control group, but there was a large difference when compared to the induced group. The administration of *K. pinnata* extracts to groups C–E led to a substantial decrease (p < 0.05) in the liver-to-body weight ratio compared to the CCl₄ group.

Table 1: Effect of ethanolic extract of *Kalochoe pinnata* on Liver-Body Weight Ratio of rats with CCL₄ -induced liver damage.

Label	Liver (g)
A	8.41 ± 0.01 ^b
B	12.47 ± 0.30 ^a
C	9.93 ± 0.06 ^c
D	7.70 ± 0.10 ^a
E	10.15 ± 0.05 ^c
F	10.46 ± 0.14 ^d
G	8.16 ± 0.05 ^a

**Effect on Full Blood Count Parameters**

Carbon tetrachloride exposure induced hematological toxicity, as reflected by significant declines in packed cell volume, hemoglobin concentration, and red blood cell count which indicates severe anemia. CCl₄ exposure led to significant ($p < 0.05$) reductions in PCV, hemoglobin concentration, and RBC count, indicating

anemia. The values ranges from 32.66 ± 10.00 to 42.33 ± 0.57 for PCV. Variations observed in red blood cell indices (PCV, Hb, RBC, MCV, MCH, and MCHC) across the experimental groups further highlight disturbances in bone marrow function and the influence of systemic oxidative stress.

Table 2: Effect of ethanolic extract of *Kalochoe pinnata* on Full Blood Count of rats with CCL₄ -induced liver damage.

Group	PCV (%)	HB (g/dl)	RBC	MCV (fL)	MCH (pg)	MCHC (g/dl)
A	38.33±0.00 ^{ab}	11.36±0.29 ^c	5.55±0.07 ^b	68.82±0.86 ^b	21.81±0.12 ^c	30.48±0.35 ^b
B	32.66±10.00 ^a	8.67±0.214 ^b	5.17±0.59 ^{ab}	67.32±0.06 ^a	14.80±0.08 ^a	22.24±0.54 ^a
C	36.66±0.57 ^{ab}	12.25±0.27 ^d	5.33±0.04 ^{ab}	68.81±0.70 ^b	23.06±0.02 ^d	33.64±0.29 ^b
D	33.66±0.57 ^a	7.84±0.76 ^a	4.93±0.07 ^a	68.82±0.08 ^b	15.86±0.11 ^b	29.76±11.6 ^{ab}
E	38.00±1.00 ^{ab}	17.3±0.56 ^f	5.51±0.02 ^b	68.84±0.06 ^b	30.77±0.20 ^g	44.82±0.13 ^c
F	42.33±0.57 ^b	15.41±0.38 ^e	6.12±0.01 ^c	68.82±0.08 ^b	24.84±0.03 ^e	36.34±0.45 ^a
G	40.33±0.57 ^b	15.40±0.65 ^e	5.62±0.04 ^c	68.77±0.39 ^a	25.98±0.67 ^f	32.11±0.74 ^{bc}

Effect on White Blood Cell Parameters

Carbon tetrachloride exposure elicited shows immune and inflammatory disruptions, as demonstrated by leukocytosis, neutrophilia, thrombocytosis, and altered leukocyte differentials, reflecting acute tissue damage and heightened systemic inflammatory stress.

Comparing negative control groups with the experimental groups to the controls shows no much difference in the parameters. The CCl₄-treated group exhibited a significant ($p < 0.05$) increase in total white blood cell count and neutrophil percentage, with a concomitant reduction in lymphocyte count, indicating inflammatory and immune dysregulation.

Tables 3: Effect of ethanolic extract of *Kalochoe pinnata* on White Blood Count Parameters of rats with CCL₄ - induced liver damage.

Grp	TWBC	Platelets	MPV (fL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
A	35.33±0.57 ^a	487.33±0.57 ^a	4.33±0.15 ^b	33.26±0.11 ^a	63.50±5.46 ^c	1.13±0.05 ^a	4.66±0.05 ^d	0.53±0.05 ^a
B	43.33±0.57 ^e	522.33±0.57 ^b	3.83±0.05 ^a	47.66±0.57 ^e	44.13±0.81 ^a	1.37±0.05 ^b	4.26±0.11 ^b	0.86±0.05 ^c
C	41.33±0.57 ^d	585.66±0.57 ^d	3.76±0.05 ^a	47.16±0.70 ^e	45.53±0.30 ^a	1.37±0.05 ^b	5.13±0.05 ^e	0.63±0.05 ^{ab}
D	37.33±0.57 ^{bc}	588.00±1.00 ^e	4.73±0.05 ^d	37.40±0.55 ^d	55.80±0.10 ^b	1.43±0.05 ^b	4.70±0.10 ^d	0.56±0.05 ^a
E	36.66±1.15 ^{ab}	487.33±0.57 ^a	4.76±0.05 ^d	35.60±0.50 ^c	57.80±0.10 ^b	1.53±0.05 ^b	3.76±0.05 ^a	1.33±0.05 ^d
F	38.33±1.15 ^c	587.33±0.57 ^e	4.53±0.15 ^c	34.40±0.36 ^b	58.80±0.10 ^b	1.76±0.11 ^c	4.26±0.05 ^b	0.76±0.05 ^c
G	37.33±0.57 ^c	500.33±0.57 ^c	4.53±0.11 ^d	35.43±0.15 ^c	60.80±0.10 ^b	1.31±0.20 ^b	4.53±0.05 ^c	0.63±0.05 ^{bc}

Values are mean ± SEM of 5 triplicates. Different superscript letters (e.g., ^a, ^b) indicate significant differences at $p < 0.05$; same letters (e.g., ^a) indicate no significant difference at $p < 0.01$ (Duncan's).

DISCUSSION

Liver-to-body weight ratio

The liver-body weight ratio was significantly higher in the CCL₄-induced negative control group (12.47 ± 0.30) compared to the normal control (8.41 ± 0.01) ($p < 0.05$), indicating hepatomegaly and severe hepatocellular injury from oxidative stress, lipid peroxidation, and reactive trichloromethyl radicals (Recknagel et al., 1989; Abdel-Moneim et al., 2020; Wang et al., 2021), consistent with reports of hepatic *Kalanchoe pinnata* extract treatment (Groups C-E and G) significantly lowered liver-body weight ratio in a dose-dependent manner. Groups D (400 mg/kg, 7.70 ± 0.10) and G (8.16 ± 0.05) showed comparable values to the control, indicating liver mass restoration and reduced damage. The highest dose (600 mg/kg) showed strong hepatoprotective effects similar to silymarin (Biswas et al., 2021; Kouakou et al., 2023), likely due to antioxidant and membrane-stabilizing properties that enhance hepatocyte regeneration and reduce oxidative stress. Post-treatment alleviated hepatomegaly but was less successful than preventative treatment, confirming the idea that ameliorative therapies can minimise liver damage but not totally avoid it (Biswas et al., 2021).

The Effects of *Kalochoe pinnata* on Full Blood Count Parameters

Changes in blood cells caused by CCL₄

Haematological parameters are essential indications of bone marrow function, oxygen transport capability, and systemic oxidative stress. In the current investigation, exposure to carbon tetrachloride (CCL₄) led to substantial decreases in haemoglobin concentration, packed cell volume (PCV), and erythrocyte count, signifying the onset of toxicant-induced anaemia. These results align with prior research indicating analogous reductions in haemoglobin (8–9 g/dL) and PCV in chemically exposed animal models (Saravanan et al., 2021; Esmat et al., 2024). The observed anaemia may result from oxidative damage to erythrocyte membranes, inhibition of erythropoiesis, and heightened red blood cell fragility and haemolysis (Adewale et al., 2022).

Kalanchoe pinnata's Haematoprotective Effects

The administration of *Kalanchoe pinnata* extract resulted in substantial enhancements in erythrocytic parameters in a dose-dependent manner. The highest dose (600 mg/kg)

notably recovered haemoglobin levels, PCV, and red blood cell counts to levels comparable to or surpassing those of the control and silymarin-treated groups. This indicates increased erythropoietic activity, stabilisation of erythrocyte membranes, and protection against oxidative haemolysis. Recent toxicological studies (Nkollo et al., 2025; Gbogbo et al., 2020) have documented analogous hematopoietic-supporting effects of antioxidant phytochemicals. These results bolster the importance of plant-derived antioxidants in preserving red blood cell integrity and bone marrow functionality (Adewale et al., 2022).

Classification of Anaemia and Erythrocyte Indices

Mean corpuscular volume (MCV) exhibited minimal variation among experimental groups, demonstrating that CCL₄-induced toxicity did not markedly affect red blood cell size, indicating a normocytic form of anaemia. Nonetheless, notable decreases in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were detected in the toxic groups, signifying hypochromic anaemia and compromised haemoglobin production (Patel et al., 2014). Treatment with *Kalanchoe pinnata* extract considerably enhanced these parameters, especially at elevated doses, presumably due to the flavonoids and phenolic substances that safeguard erythrocytes from oxidative damage and facilitate haemoglobin formation (Biswas et al., 2021; Kumar et al., 2019).

Responses of the immune system and inflammation

Exposure to CCL₄ caused significant changes in the immune system, such as leukocytosis, neutrophilia, and thrombocytosis. These changes are signs of acute inflammation and immune system activation. The increased total white blood cell (TWBC) counts and platelet levels found in this investigation align with prior findings in toxicant-induced models (Abdel-Moneim et al., 2020; Patel et al., 2014; Esmat et al., 2024). These alterations correlate with oxidative stress-induced cytokine release, leukocyte mobilisation, and tissue damage, (Iboyi et al., 2026).

Kalanchoe pinnata's effects on the immune system

Kalanchoe pinnata extract treatment led to a dose-dependent restoration of white blood cell and platelet parameters. Higher doses (400 and 600 mg/kg)

dramatically decreased TWBC numbers, re-established neutrophil-lymphocyte equilibrium, and normalised monocyte, eosinophil, and basophil levels. Platelet parameters, such as mean platelet volume (MPV), also got better, which means that platelet activation and stress on the blood vessels were lower. These findings indicate that the extract inhibits excessive inflammatory signalling and re-establishes immunological homeostasis.

Kalanchoe pinnata's immunomodulatory activity is probably due to its high levels of flavonoids, phenolic acids, and other antioxidant compounds, which are known to pick up reactive oxygen species and stop the production of pro-inflammatory cytokines (Omodamiro & Eze, 2021; Wang et al., 2022; Zhang et al., 2020). Similar results have been documented in research illustrating the anti-inflammatory and immune-stabilizing properties of plant-derived antioxidants (Gabriel-Brisibe et al., 2025; Okoye et al., 2023).

The results of this study show that *Kalanchoe pinnata* has strong immunomodulatory and haematoprotective capabilities against toxicity caused by CCl₄. The extract efficiently alleviates anaemia, restores erythrocyte integrity, and modulates immunological responses, presumably via its antioxidant, anti-inflammatory, and membrane-stabilizing properties. These findings substantiate the therapeutic efficacy of *Kalanchoe pinnata* in the management of toxin-induced haematological and immunological diseases.

CONCLUSION

The results indicate that *Kalanchoe pinnata* possesses considerable haematological and immunological protective properties against CCl₄-induced toxicity. The extract significantly diminished hepatic enlargement, enhanced erythrocyte parameters, and regulated immunological responses, thus alleviating anaemia and inflammation. The effects are mostly ascribed to its antioxidant and phytochemical constituents, endorsing its prospective application as a natural medicinal agent in the management of toxin-induced haematological and immunological diseases. This investigation substantiates the therapeutic efficacy of *Kalanchoe pinnata* in alleviating CCl₄-induced hepatic injury and systemic toxicity. The extract safeguards hepatic architecture and functionality while also improving haematological stability and immunological equilibrium, so corroborating its historical use as a medicinal plant and indicating its prospective utility in the treatment of toxin-induced organ injury.

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