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Evaluation of Platelet and Haemostatic Biomarkers of Methanol Leaves Extract of Chromolaena Odorata In Cyclophosphamide-Induced Thrombocytopenia in Wistar Rats

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Abstract

Thrombocytopenia is a clinically important haematological disorder, but platelet count alone does not describe its effects on platelet biology and haemostasis. This study evaluated the effect of methanol leaf extract of Chromolaena odorata on platelet count, platelet indices (mean platelet volume, MPV; platelet distribution width, PDW; plateletcrit, PCT; platelet large cell ratio, P-LCR), von Willebrand factor (vWF), and fibrinogen in cyclophosphamide-induced thrombocytopenia in Wistar rats. Cyclophosphamide was used solely to induce thrombocytopenia. Acute oral toxicity (LD50) was first determined using Lorke's metho. Twenty-five Wistar rats were allocated to five groups (n=5): normal control, negative control (cyclophosphamide only), and three groups administered cyclophosphamide with the extract orally at 125, 250, or 500 mg/kg respectively for two weeks. Platelet count and indices were measured using an automated haematology analyser, and vWF and fibrinogen were measured by ELISA. Data were analysed by one-way ANOVA ($p<0.05$). No mortality was observed up to 5000 mg/kg. Significant group differences were observed for platelet count, PDW, PCT, P-LCR, and vWF, but not for MPV or fibrinogen. Cyclophosphamide reduced platelet count, PCT, and vWF and increased PDW and P-LCR. It has been seen that in all the extract-treated groups there is higher platelet count and PCT and lower PDW than the negative control; vWF was highest at 250 mg/kg, and P-LCR was lowest at 125 mg/kg. The extract restored platelet count and returned platelet-turnover indices and vWF toward normal values in cyclophosphamide-induced thrombocytopenic rats. Findings provide experimental evidence that methanol leaves extract of C. odorata possesses promising thrombocytopoietic properties supporting further investigation.

Keywords: Chromolaena odorata; thrombocytopenia; cyclophosphamide; platelet indices; von Willebrand factor; fibrinogen; Wistar rats.

1. Introduction

Childhood Thrombocytopenia is a common and clinically important haematological disorder. Platelet count is most familiar laboratory marker, yet it provides an incomplete description of platelet biology. Platelet indices, namely mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and platelet large cell ratio (P-LCR), provide additional information on platelet turnover and bone marrow activity (Senthil Nathan et al., 2024; Moroi et al., 2020). Von Willebrand factor (vWF) is synthesised by endothelial cells and megakaryocytes, it mediates platelet adhesion, and is sensitive to endothelial oxidative injury. Fibrinogen, which is synthesised in the liver, remains comparatively stable unless hepatic synthetic function is impaired or systemic inflammation is present (Sadler, 1998; Ruggeri, 2007; Weisel and Litvinov, 2017). Together, platelet indices, vWF, and fibrinogen reflect platelet production, endothelial function, and hepatic synthesis. These three processes cannot be distinguished by platelet count alone, and they carry different implications for bleeding risk and recovery (Chornenki et al., 2022; Carson and Stanworth, 2017).

Pharmacological options for treating thrombocytopenia remain limited, which has stimulated interest in plant-derived extracts as platelet-supportive agents. Such extracts are commonly evaluated in animal models of cyclophosphamide-induced thrombocytopenia, although platelet indices are rarely measured alongside both vWF and fibrinogen (Gholamnezhad et al., 2023; Munir et al., 2022; Sailor et al., 2021).

Chromolaena odorata (Asteraceae), commonly called Siam weed, is known in Nigeria as Ewe Awolowo (Yoruba), Obirakara, and Olorohuru (Ngozi and Osuji, 2014). The plant has an ethnomedicinal reputation for haemostasis and wound healing and is rich in flavonoids and phenolics with antioxidant activity (Vijayaraghavan et al., 2017; Anyasor et al., 2011; Femi et al., 2022). Because the myelosuppressive toxicity of cyclophosphamide and endothelial vWF release are both sensitive to oxidative stress, antioxidant activity is a plausible mechanistic link to the outcomes studied here, although oxidative stress was not measured in this study (Korkmaz et al., 2007; Emadi et al., 2009).

An aqueous extract of C. odorata has been shown to improved platelet recovery in a busulfan-induced thrombocytopenia model (Paul et al., 2018). Aqueous extract has also been studied against doxorubicin-induced toxicity, with a further report of platelet indices in that model whose journal could not be independently confirmed (Ikewuchi et al., 2021). No study identified so far has reported vWF for any C. odorata extract in any model, which is the principal novelty of this work. The fibrinogen finding is novel only in the context of thrombocytopenia, since fibrinogen has been measured previously in healthy rats.

This study therefore evaluated whether methanol leaf extract of C. odorata modulates platelet count, platelet-turnover indices, vWF, and fibrinogen in cyclophosphamide-induced thrombocytopenia in Wistar rats. We hypothesised that the extract would restore platelet count, normalise platelet-turnover indices, and modulate vWF and fibrinogen. Cyclophosphamide was used solely to induce thrombocytopenia; the study did not aim to model or treat cancer.

2. Methodology

2.1 Study design

This was a randomised controlled experimental study in Wistar rats evaluating the effect of methanol leaf extract of C. odorata on platelet count, platelet indices (MPV, PDW, PCT, and P-LCR), von Willebrand factor, and fibrinogen in cyclophosphamide-induced thrombocytopenia. The study comprised two phases: an acute oral toxicity (LD50) study in a separate cohort of 12 rats, followed by a main experiment in 25 rats allocated to five groups (normal control, negative control, and three extract doses of 125, 250, and 500 mg/kg). In the main experiment, thrombocytopenia was induced with cyclophosphamide, and outcomes were measured at the end of a two-week oral dosing period.

2.2 Study Location

This study was carried out in the Department of Haematology, School of Medical Laboratory Sciences, in collaboration with Haematology Department, Usmanu Danfodiyo University Teaching Hospital (UDUTH), and Animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

2.3 Ethical Approval

The study followed applicable ethical guidelines for research involving animal subjects, and ethical approval was obtained beforehand from the Research Ethics Committee of Usmanu Danfodiyo University Sokoto with Ref/ID No: HBREC/26/107.

2.4 Plant material and extraction

Fresh C. odorata leaves were collected from a medicinal herbal farm in Ilorin, Kwara State, Nigeria. The plant was authenticated at the Herbarium Unit, Department of Pharmacology and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto (UDUS), Nigeria (voucher no. PCG/UDUS/ASTE/0008). The leaves were shade-dried for 7 days and pulverised, and approximately 400 g of the powdered material was macerated in 3000 mL methanol for 48 hours, filtered, and evaporated to dryness at 37°C, yielding 61 g of crude extract (15.3% yield). The dried extract was reconstituted in normal saline immediately before oral administration.

2.5 Sample size

Group size was determined using the Resource Equation method (Mead, 1988; Arifin and Zahiruddin, 2017),

yielding a minimum of 25 animals for the main experiment (5 groups × n=5).

2.6 Acute oral toxicity (LD50)

Acute oral toxicity of methanol leave extract of C. odorata was determined in two (2) separate phases of 12 Wistar rats using Lorke's (1983) method. (Phase I: 10, 100, and 1000 mg/kg, n=3/group; Phase II: 1600, 2900, and 5000 mg/kg, n=1/group), with observation for 24 hours.

2.7 Experimental animals

Thirty-seven Wistar rats weighing 200–220g were obtained from the Animal House Unit, Ahmadu Bello University, Zaria (12 for the toxicity study and 25 for the main experiment) and allowed tp acclimatised for two weeks with free access to food and water.

2.8 Induction of Thrombocytopenia

Thrombocytopenia was induced by intraperitoneal administration of cyclophosphamide at a dose of 20mg/kg body weight for four (4) days in order to establish a thrombocytopenic model (Gauer and Whitaker, 2022).

2.9 Confirmation of Thrombocytopenia

After final day of cyclophosphamide administration blood sample were collected into EDTA anticoagulated tubes for haematological analysis. Platelets count was determined using an automated hematology analyser. Successful induction of thrombocytopenia was confirmed by a significant reduction in platelet count blow 100x109/l relative to the normal control group of platelets count above100x109/l (Yang et al., 2025; Prompetcharaet al., 2024; Elbeshlawy et al., 2022).

2.10 Experimental groups and treatment

Animals were assigned to five groups of five: (1) normal control, which received no induction or treatment; (2) negative control, which received cyclophosphamide only without treatment; and 3, 4 and 5 treatment groups, which received cyclophosphamide together with methanol leaf extract of C. odorata at 125, 250, or 500 mg/kg respectively, as shown in the table below.

Table 1 Experimental design

Group	N	Induction (4 days)	Intervention	Route	Duration
1 — Normal control	5	None	No intervention	Oral	Two weeks
2 — Negative control	5	Cyclophosphamide (20mg/kg)	No intervention	Oral	Two weeks
3 — Test 1	5	Cyclophosphamide (20 mg/kg)	*C. odorata* extract, 125 mg/kg	Oral	Two weeks
4 — Test 2	5	Cyclophosphamide (20 mg/kg)	*C. odorata* extract, 250 mg/kg	Oral	Two weeks
5 — Test 3	5	Cyclophosphamide (20 mg/kg)	*C. odorata* extract, 500 mg/kg	Oral	Two weeks

2.11 Sample collection

Twenty-four hours after the final dose, animals were anaesthetised with ketamine (75 mg/kg), and blood was collected by cardiac puncture into K3-EDTA tubes (for platelet indices) and into plain tubes for the ELISA assays of fibrinogen and Von-Willebrand factor.

2.12 Laboratory Analysis

2.12.1 Platelet count and platelet indices

Platelet count (PLT), MPV, PDW, PCT, and P-LCR were determined using a five-part automated haematology analyser (Mythic 60, ORPHEE SA, Switzerland), based on the electrical impedance (Coulter) principle, following the method of Ochei and Kolhatkar (2003).

2.12.2 Von Willebrand factor

Plasma vWF antigen was determined using a shanghai ideal medical technology Co. LTD ELISA kit (enzyme-linked immunosorbent assay) stored at temperature 2-8 OC, LOT No 202601 and expiring date 01/2027 following Ochei and Kolhatkar (2003).

2.12.3 Fibrinogen

Plasma fibrinogen was determined using shanghai ideal medical technology Co. LTD Fibrinogen ELISA kit stored at temperature 2-8O C, LOT No 202601 and expiring date 01/2027 following Ochei and Kolhatkar (2003).

2.13 Data analysis

Data were analysed using IBM SPSS version 25 and are presented as mean ± SD. Differences between groups were assessed by one-way ANOVA, with $p<0.05$ considered significant.

3. Results

The result of acute toxicity study LD50, the mean and standard deviation of platelets count and indices as well as that of fibrinogen and Von-Willebrand factor of all the experimental animal including their respective controls in this study were shown in table 2 to 4. The Post Hoc analysis indicating differences between the groups are equally presented.

Table 2. Oral Acute Toxicity (LD_{50}) Studies of Methanol Leaves Extract of *C. Odorata*

Groups	Doses (mg/kg)	Observation Period	Behavioral Changes	Mortality
Phase 1		24 hours		
1: 3 rats	10		None	None
2: 3 rats	100		None	None
3: 3 rats	1000		None	None
Control	0			
Phase II				
1: 1 rat	1600		None	None
2: 1 rat	2900		None	None
3: 1 rat	5000		None	None

The LD50 of *C. Odorata* leaves extract was found to be greater than 5000 mg/kg (> 5000 mg/kg) body weight at the administered dose.

Table 2. shows the results of the acute oral toxicity study of the methanol leaf extract of C. odorata in cyclophosphamide-induced thrombocytopenic Wistar rats. The acute toxicity study was conducted in two consecutive phases to evaluate the safety profile of the methanol leaf extract.

Table 3. Effect of Methanol Leaves Extract of *C. Odorata* on Platelet Count and Platelet Indices in Cyclophosphamides Induced Thrombocytopenic Wistar Rats

Parameter	Normal control (N=5)	Negative control (N=5)	125mg/kg (N=5)	250mg/kg (N=5)	500mg/kg (N=5)
PLT ($\times10^9$/L)	681.60±142.86[a]	58.00±13.53[b]	810.50±85.56 [a]	694.25±143.52 [a]	1000.50±70.00 [a]
PDW (fL)	8.60±1.09 [a]	14.43±1.23 [b]	8.70±0.57 [a]	8.78±1.01 [a]	9.45±1.91 [a]
MPV (fL)	7.34± 0.64 [a]	6.87±0.55 [a]	7.20±0.42 [a]	7.40±0.65 [a]	7.35±0.50 [a]
P-LCR (%)	8.16±1.34 [a]	13.07±1.60 [b]	7.10±1.41 [a]	11.95±1.11 [b]	9.10±5.09 [a]
PCT (%)	0.556±0.153 [a]	0.039±0.006 [b]	0.730±0.113 [a]	0.628±0.314 [a]	0.820±0.127 [a]

Data are expressed as Mean ± SD (Mean ± Standard Deviation). Values bearing difference superscript on the same row differ significantly. Platelet count (PLT) ($\times10^9$/L), Platelet distribution width (PDW), Femto Liter (fL), Mean platelet volume (MPV), Platelet large cell ratio (P-LCR), percentage (%), and Plateletcrit (PCT).

Table 3. Shows Effect of Methanol Leaves Extract of C. Odorata on Platelet Count and Platelet Indices in Cyclophosphamides Induced Thrombocytopenic Wistar Rats,

Platelet count (PLT) decreased markedly in the negative control (58.00±13.53 ×109/L) relative to the normal control (681.60±142.86 ×109/L), confirming successful induction of thrombocytopenia. Treatment increased PLT, PDW, PCT AND P-LCR in all the treatment groups with $p<0.05$. However, MPV showed no significant variation across groups (6.87–7.40 fL; overall $p=0.793$).

Table 4: Effect of Methanol Leaves Extract of *C. Odorata* on Von Willebrand and fibrinogen in Cyclophosphamides Induced Thrombocytopenic Wistar Rats

Parameter	Normal control (N=5)	Negative control (N=5)	125mg/kg (N=5)	250mg/kg (N=5)	500mg/kg (N=5)
vWF (IU/dL)	549.21±78.29[a]	451.41±32.88[b]	540.40±47.72 [a]	617.94±40.38 [a]	571.55±51.43 [a]
Fibrinogen (g/L)	37.74±3.08 [a]	33.66±2.25 [a]	38.81±1.16 [a]	39.75±4.31 [a]	36.16±0.70 [a]

Data are expressed as Mean ± SD (Mean ± Standard Deviation). Values bearing difference superscript on the same row differ significantly. Von-Willebrand Factor (vWF) (International Unit/deciLitre), and Gram per Litre (g/L).

Table 4: Shows Effect of Methanol Leaves Extract of C. Odorata on Von Willebrand and fibrinogen in Cyclophosphamides Induced Thrombocytopenic Wistar Rats

Von Willebrand factor (vWF) differed significantly across groups (p=0.039). there was a significant decreased in vWF among the negative control (451.41±32.88 IU/dL) relative to the normal control (549.21±78.29 IU/dL) and increased with treatment, most notably at 250 mg/kg (617.94±40.38 IU/dL). Fibrinogen level on the other hand showed no significant difference across groups (p=0.187), varying minimally from 33.66±2.25 to 39.75±4.31 g/L.

4. Discussion

This present study demonstrated that cyclophosphamide administration caused a significant alteration in haematological parameters, confirming successful induction of bone marrow suppression and thrombocytopenia. Cyclophosphamide is a well-known cytotoxic agent that suppresses rapidly dividing haematopoietic cells, leading to reductions in blood cell production (Basu et al., 2025). However, treatment with methanol leaf extract of C. Odorata significantly improved most of the altered pletelets parameters, indicating that the extract possesses haematopoietic and myeloprotective properties (Sheneni et al., 2026).

No signs of toxicity or mortality were observed in the experimental animals at the tested doses, suggesting that the methanol leaf extract of C. Odorata extract appears to be non-toxic under the conditions of this acute oral toxicity study and is safe for consumption, this is in agreement with work carried out by Sheneni et al., (2026).

The marked decrease in platelet count (PLT) in the negative control group as shown in table 3 confirms that cyclophosphamide induced thrombocytopenia in this model, in keeping with its myelosuppressive action on rapidly dividing haematopoietic progenitors, including megakaryocytes (Emadi et al., 2009; Hoffman et al., 2022). Extract treatment of C. Odorata restored platelet count to the normal control level or above at all three doses (Table 3). The increase was not strictly dose-ordered, with the 250 mg/kg group closest to the normal control and the 500 mg/kg group highest. The data therefore support restoration of platelet count by the extract but do not establish a clear dose-response relationship. A plausible explanation is that the antioxidant flavonoids and phenolics of the extract limit oxidative injury to marrow progenitors (Emadi et al., 2009; Vijayaraghavan et al., 2017). However, oxidative stress markers and megakaryocyte activity were not measured directly, so the proposed mechanism remains inferential.

The marked elevation of PDW in the negative control reflects the size heterogeneity that accompanies bone marrow stress and premature release of immature platelets in drug-induced thrombocytopenia. Upon treatment with methanol extract of C. Odorata it can be seen that PDW was restored to normal in all three treatment groups as seen in table 3. It is consistent with restored regulation of platelet maturation rather than a simple increase in circulating platelet number (Bath and Butterworth, 1996; Senthil Nathan et al., 2024). PCT, which reflects total circulating platelet mass, changed in the same direction as platelet count, consistent with restored overall platelet mass. The stability of MPV across groups agrees with reports that MPV responds less sharply than PDW or PCT to acute changes in platelet production, making it a comparatively insensitive marker of this type of recovery (Hoffman et al., 2022).

The P-LCR pattern was the least consistent of the platelet indices. P-LCR was elevated in the negative control and lowest at 125 mg/kg dose group. At 250 mg/kg it remained close to the negative control, whereas the 500 mg/kg value (9.10%) was close to the normal control but showed wide variability (SD 5.09%). The data therefore do not show a consistent dose-related change in P-LCR and cannot distinguish partial normalisation at 250 and 500 mg/kg from the absence of a reliable effect. P-LCR reflects the proportion of large platelets in circulation, and platelet populations recovering from marrow suppression that may pass through a transient phase of increased large-platelet output before settling to a more uniform size distribution. One plausible interpretation is that the higher doses represent a different point along a platelet-recovery trajectory. This interpretation is speculative and cannot be distinguished from assay variability, a true non-monotonic dose relationship, or chance without a recovery time-course, which this study did not include.

The decrease in vWF in the negative control as seen in table 4 is consistent with cyclophosphamide-associated endothelial disturbance, given the established sensitivity of vWF to endothelial oxidative injury (Sadler, 1998; Ruggeri, 2007). The increase in vWF with treatment, greatest at 250 mg/kg, is consistent with an endothelial-protective effect of the extract, but does not follow a monotonic dose-response. Antioxidant activity of the extract limiting endothelial oxidative injury is a plausible mechanistic hypothesis, in line with reports that flavonoid-rich extracts support endothelial function (Vijayaraghavan et al., 2017). However, oxidative stress biomarkers and endothelial function were not measured directly, so the mechanism remains inferential rather than demonstrated.

The stability of fibrinogen across all groups (Table 4) indicates that hepatic synthetic function and the final common coagulation pathway were not meaningfully disturbed by either cyclophosphamide or extract treatment in this model. This is consistent with the general observation that fibrinogen levels remain stable in the absence of severe systemic inflammation, hepatic dysfunction, or a consumptive process such as disseminated intravascular coagulation (Weisel and Litvinov, 2017; Asakura, 2021). Considered alongside the companion article's finding of an unchanged PT, this indicates that the action of the extract in this model is selective for platelet-production and endothelial pathways rather than a broad effect on hepatic coagulation-protein synthesis (Asakura, 2021).

5. Conclusion

Methanol leaf extract of C. odorata is safe for consumption as evident by the acute oral toxicity result. It has also proven to restored platelet count and normalised platelet-turnover indices (PDW, PCT) across the dose range tested and increased the endothelial biomarker vWF, most clearly at 250 mg/kg, in cyclophosphamide-induced thrombocytopenic rats, while MPV and fibrinogen remained unaffected.

Finally, the findings provide experimental evidence that methanol leaves extract of C. odorata possesses promising thrombocytopoietic properties and may be beneficial in ameliorating cyclophosphamide-induced thrombocytopenia.

6. Recommendations

1. Conduct sub-chronic and chronic toxicity studies, including histopathological assessment, to establish long-term safety.

2. Conduct molecular studies to determine how the extract affects platelet and clotting factor production.

3. Assess platelet activation markers, inflammatory cytokines, thrombopoietin, oxidative stress markers, and bone marrow histology to better understand the mechanism of action.

7. Limitations

1. The group size was small and fixed (n=5) and was determined by a resource-equation animal-welfare justification rather than a power calculation for a pre-specified effect size. This is particularly relevant to the non-monotonic P-LCR and vWF patterns, where a larger sample might clarify whether they reflect a true dose-dependent ceiling or threshold effect or assay and biological variability.

2. Only acute toxicity was assessed; sub-chronic and chronic toxicity profiles remain unknown.

8. Contribution to Knowledge

To the best of our knowledge, based on the literature searched for this manuscript, this study provides the first evaluation of von Willebrand factor and fibrinogen for any Chromolaena odorata extract in a thrombocytopenia model. It also extends the existing platelet-index literature on C. odorata, previously reported only for an aqueous extract against doxorubicin, to a methanol extract tested against cyclophosphamide-induced thrombocytopenia.

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Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

All authors contributed to the conception, conduct, analysis, interpretation and preparation or review of the manuscript according to their respective roles.

References

1. Anyasor, G. N., Aina, D. A., Olushola, M., and Aniyikaye, A. F. (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of Chromolaena odorata. Annals of Biological Research, 2(2): 441–451.

2. Arifin, W. N., and Zahiruddin, W. M. (2017). Sample size calculation in animal studies using resource equation approach. Malaysian Journal of Medical Sciences, 24(5): 101–105.

3. Asakura, H. (2021). Diversity of disseminated intravascular coagulation and selection of appropriate treatments. International Journal of Hematology, 113: 10–14.

4. Basu, S., Mukherjee, S., Chakraborty, S., & Dey, A. (2025). Role of traditional Indian plants on treating myelosuppression caused by chemotherapy with special reference to cyclophosphamide. The Nucleus, 68(1), 195-211.

5. Bath, P. M. W., and Butterworth, R. J. (1996). Platelet size: measurement, physiology and vascular disease. Blood Coagulation & Fibrinolysis, 7(2): 157–161.

6. Carson, J. L., and Stanworth, S. J. (2017). Anemia and bleeding in thrombocytopenic patients. Blood, 130(10): 1178–1179.

7. Chornenki, N. L. J., Fralick, M., and Sholzberg, M. (2022). International normalized ratio and activated partial thromboplastin time testing. Canadian Medical Association Journal, 194(33): e1135.

8. Emadi, A., Jones, R. J., and Brodsky, R. A. (2009). Cyclophosphamide and cancer therapy: Mechanisms of toxicity and hematologic suppression. Clinical Cancer Research, 15(24): 7855–7861.

9. Femi, O., Kolawole, O., and Opeyemi, I. (2022). Biological activities of Chromolaena odorata: A mechanistic review. South African Journal of Botany, 144: 44–57.

10. Gholamnezhad, Z., Rouki, V., Rezaee, R., and Boskabady, M. H. (2023). Medicago sativa ameliorated cyclophosphamide-induced thrombocytopenia and oxidative stress in rats. Toxin Reviews, 42(2): 527–536.

11. Hoffman, R., Benz, E. J., Silberstein, L. E., Heslop,

H. E., Weitz, J. I., Anastasi, J., Salama, M. E., and Abutalib, S. A. (Eds.). (2022). Hematology: Basic Principles and Practice (8th ed.). Elsevier.

12. Ikewuchi, C. C., Ikewuchi, J. C., Ifeanacho, M. O., Jack, D. P., Ikpe, C. N., Ehiosun, S., and Ajayi, T. B. (2021). Protective effect of aqueous leaf extracts of Chromolaena odorata and Tridax procumbens on doxorubicin-induced hepatotoxicity in Wistar rats. Porto Biomedical Journal, 6(6): e143.

13. Korkmaz, A., Topal, T., and Oter, S. (2007). Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell Biology and Toxicology, 23(5): 303–312.

14. Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54(4): 275–287.

15. Matthew, L. O., and Osime, E. O. (2019). Haemostatic properties of Vernonia amygdalina and Chromolaena odorata leaf extracts using Wistar rat model. Universa Medicina, 38(2): 124–131.

16. Mead, R. (1988). The Design of Experiments: Statistical Principles for Practical Applications. Cambridge University Press.

17. Moroi, M., Farndale, R. W., and Jung, S. M. (2020). Activation-induced changes in platelet surface receptor expression and the contribution of the large-platelet subpopulation to activation. Research and Practice in Thrombosis and Haemostasis, 4(2): 285–297.

18. Munir, S., Liu, Z.-W., Tariq, T., Rabail, R., Kowalczewski, P. Ł., Lewandowicz, J., Blecharczyk, A., Abid, M., Inam-Ur-Raheem, M., and Aadil, R. M. (2022). Delving into the therapeutic potential of Carica papaya leaf against thrombocytopenia. Molecules, 27(9): 2760.

19. Ngozi, N., and Osuji, T. (2014). Personal communication on the relevance and indigenous use of medicinal plants.

20. Ochei, J., and Kolhatkar, A. (2003). Medical Laboratory Science: Theory and Practice. McGraw-Hill.

21. OECD (2001). Guidelines for the Testing of Chemicals: Acute Oral Toxicity – Acute Toxic Class Method, No. 423.

22. Okoroiwu, H. U., Atangwho, I. J., Uko, E. K., and Maryann, O. I. (2016). Haemostatic property of Chromolaena odorata leaf extracts: in vitro and in vivo evaluation in Wistar rats. Journal of Biological Research – Bollettino della Società Italiana di Biologia Sperimentale, 89(2).

23. Paul, T. S., Das, B. B., Ingale, S. P., Killedar, N., and Apte, K. G. (2018). Oral intake of polyphenols of Chromolaena odorata: a perspective in peptic ulcer, thrombocytopenia, and heparin-induced bleeding diathesis in rodent model. Pharmacognosy Research, 10(4): 426–431.

24. Ruggeri, Z. M. (2007). The role of von Willebrand factor in thrombus formation. Thrombosis Research, 120(Suppl 1), S5–S9.

25. Sadler, J. E. (1998). Biochemistry and genetics of von Willebrand factor. Annual Review of Biochemistry, 67: 395–424.

26. Sailor, G., Hirani, K., Parmar, G., Maheshwari, R., Singh, R., and Seth, A. K. (2021). Platelet augmentation potential of polyherbal formulation in cyclophosphamide-induced thrombocytopenia in Wistar rats. Folia Medica, 63(1): 67–73.

27. Senthil Nathan, S., Varadaraj, P., Nallusamy, G., and Reddy, K. S. S. (2024). The significance of platelet indices in the evaluation of thrombocytopenia. Cureus, 16(7): e65756.

28. Sheneni, V. D., Anorue, E. C., Joshua, P. E., Ugwuene, F. O., & Agwu, L. O. (2026). Amelioration of cyclophosphamide-induced myelosuppression by the methanol fraction of Talinum fruticosum. Drug and Chemical Toxicology, 1-22.

29. Vijayaraghavan, K., Rajkumar, J., Bukhari, S. N. A., Al-Sayed, B., and Seyed, M. A. (2017). Chromolaena odorata: a neglected weed with a wide spectrum of pharmacological activities. Molecular Medicine Reports, 15(4): 1007–1016.

30. Weisel, J. W., & Litvinov, R. I. (2017). Fibrin formation, structure and properties. Sub-Cellular Biochemistry, 82: 405–456.