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Optimal dose of *Moringa oleifera Lam* for hematopoietic recovery in aluminum-induced hematotoxicity

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ABSTRACT

Background: Aluminum-induced hematotoxicity disrupts hematopoiesis and leads to anemia primarily through oxidative stress mechanisms. Natural antioxidants such as Moringa oleifera have shown potential protective effects; however, the optimal therapeutic dose remains unclear.

Objective: This study aimed to evaluate the dose-dependent effects of Moringa oleifera leaf extract and determine the optimal dose to improve hematological parameters in aluminum-induced hematotoxicity.

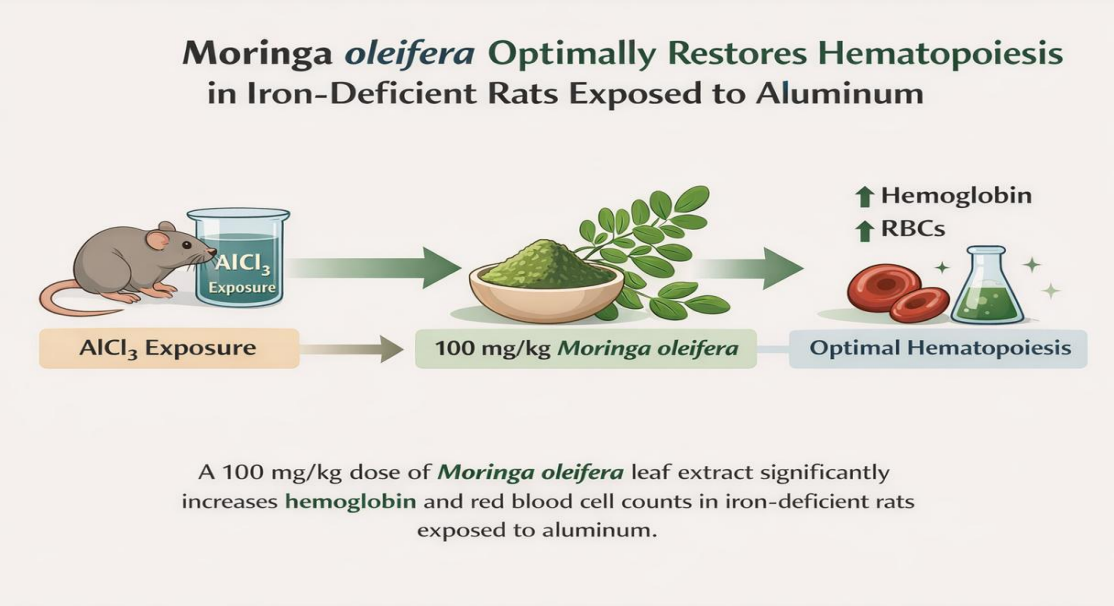
Methods: Twenty-four male Wistar rats were randomly assigned into four groups (n = 6): control, $AlCl_3$ + 200 mg/kg Moringa oleifera, $AlCl_3$ + 100 mg/kg Moringa oleifera, and Moringa oleifera (200 mg/kg) only. Hematological parameters, including hemoglobin concentration, red blood cell count, hematocrit, and differential leukocyte counts, were measured over 14 days. Statistical analysis was performed using one-way ANOVA, with $p \leq 0.05$ considered significant.

Results: Treatment with Moringa oleifera significantly improved hematological parameters compared to the $AlCl_3$-exposed group ($p \leq 0.05$). The 100 mg/kg dose demonstrated superior efficacy, with higher hemoglobin (12.00 g/dL), red blood cell count (7.23×10^6/µL), and hematocrit (41.50%) compared to the 200 mg/kg dose. Additionally, an increase in lymphocyte percentage was observed. The dose-response relationship indicated a hormetic effect, where moderate dosing produced greater benefits than higher dosing.

Conclusion: Moringa oleifera exhibits significant protective effects against aluminum-induced hematotoxicity, with 100 mg/kg identified as the most effective dose. These findings highlight its potential as a natural therapeutic agent for improving hematological health under toxic conditions

Novelty of the study: This study identifies an optimal dose (100 mg/kg) of Moringa oleifera for hematological recovery in aluminum-induced toxicity and provides evidence of a hormetic dose-response effect in hematopoietic protection.

Key Words: Aluminum chloride; anemia; dose–response; erythropoiesis; *Moringa oleifera*; hematopoietic recovery; bioactive dose.



Graphical Abstract: Optimal Dose of Moringa oleifera Lam for Hematopoietic Recovery in Aluminum-Induced Hematotoxicity

INTRODUCTION

Iron Deficiency Anemia (IDA) is a nutritional disorder that occurs in more than 30% of the world's population and is a major health concern in society, particularly in children and pregnant women [1]. IDA causes hypoxia, cognitive impairment, and immune system depression in the body [2]. Iron salts are considered the first-line treatment for patients with Iron Deficiency Anemia. However, iron salts are not always effective in treating anemia due to gastrointestinal side effects and low bioavailability. Therefore, interest in functional foods, which are substances that provide health benefits to humans in addition to providing nutritional value, as an alternative to iron salts in treating anemia, is increasing [3-4]. In this context, *Moringa oleifera* Lam. (Moringaceae), a plant with potential as a functional food due to its nutritional and phytochemical properties, has been identified as an alternative to iron salts for the treatment of anemia [5-6]. Recent studies show that M. oleifera leaves contain iron, vitamin C, and amino acids that can enhance duodenal iron absorption [7-8]. In addition to these nutrients, the plant contains high levels of bioactive compounds, including glucosinolates, isothiocyanates, and polyphenols such as quercetin and kaempferol [9]. These compounds have shown significant antioxidant potential in maintaining the stability of the erythrocyte (RBC) cell membrane. They also show protective effects against oxidative stress and hemolysis [10-11]. There is also emerging evidence that bioactive compounds in M. oleifera may modulate erythropoiesis by influencing erythropoietin (EPO) signaling pathways. However, the exact mechanisms by which this is achieved are poorly understood [6,12]. This is particularly significant in cases of environmental toxins such as aluminum chloride ($AlCl_3$), which causes oxidative stress and impairs bone marrow and hematopoiesis [13-14]. In such situations, the antioxidant and cytoprotective effects of M. oleifera may help maintain hematological homeostasis. Despite the recognized nutritional and therapeutic value of M. oleifera, a significant knowledge gap exists on the 'bioactive dosage,' which refers to the exact amount of M. oleifera extract that can bring about beneficial effects on hematological parameters in the absence of side effects [4, 15]. This study aims to investigate the dose-response effects of methanolic *Moringa oleifera* leaf extract on Wistar rats with aluminum chloride ($AlCl_3$)-induced hematotoxicity to determine the optimal bioactive dose that can promote erythropoiesis and improve blood parameters.

MATERIALS AND METHODS

Plant Collection and Extraction: Fresh leaves of Moringa oleifera were hand-picked from the Botanical Garden of Khartoum State. The leaves were identified by experts at the University of Khartoum. The leaves were dried at 25 °C, then ground into a fine powder [16]. The leaves were extracted using the Soxhlet method with 80% methanol as the extracting agent for 6 hours, which facilitated the solubilization of the phenolic and flavonoid compounds present in the leaves [17-18]. The filtered extract was further dried at 40 °C, then stored at 4 °C for future use [19-20].

$$\text{Yield } (\%) = \frac{\text{Weight of dried extract}}{\text{Weight of initial leaf powder}} \times 100$$

Experimental Animals: Rats were randomly assigned to four groups (n = 6):

1. Control (CG): Received no treatment.
2. Group 1 (G1): $AlCl_3$ (0.5 mg/kg) + *Moringa oleifera* extract (200 mg/kg).
3. Group 2 (G2): $AlCl_3$ (0.5 mg/kg) + *Moringa oleifera* extract (100 mg/kg**).**
4. Group 3 (G3): *Moringa oleifera* extract alone (200 mg/kg).

The doses of *Moringa oleifera* (100 and 200 mg/kg) were selected based on prior studies demonstrating efficacy and safety in rodent models, with 100 mg/kg representing a moderate bioactive dose and 200 mg/kg representing a higher dose to evaluate dose-dependent effects and identify the optimal bioactive dosage for hematopoietic recovery [7,14]. Treatments were administered daily via oral gavage for 14 days [21].

Animals were assigned to experimental groups using a computer-generated randomization schedule to minimize selection bias. Investigators responsible for treatments and sample collection were blinded to group allocation to reduce observer bias and enhance methodological rigor.

Hematological Analysis: Blood samples (2 mL) were collected via the retro-orbital plexus into EDTA-coated tubes at the end of Week 1 and Week 2 [22]. Hematological parameters measured included hemoglobin (Hb), red blood cell (RBC) count, hematocrit (Hct), mean corpuscular volume (MCV), and lymphocyte percentage. Analyses were performed using a Sysmex 8999 auto-analyzer, following the manufacturer's instructions to ensure accuracy and reproducibility.

Statistical Analysis: Data were expressed as mean ± standard error (SE). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) according to standard procedures [23], followed by an appropriate post-hoc test to determine pairwise differences when overall significance was detected. A p-value < 0.05 was considered statistically significant. Statistical significance markers were clearly indicated in all figures and figure legends to denote differences between treatment groups.

To enhance methodological rigor, animals were randomly assigned to experimental groups using a computer-generated randomization schedule to minimize selection bias. Investigators responsible for data collection and hematological measurements were blinded to group allocation to reduce observer bias. These procedures were implemented to improve reproducibility and ensure transparency in the experimental design.

Table 1. The effect of *M.oleifera* methanolic extract on hematological parameters of rats after one week of treatment.

Parameters Groups	WBC ($\times10^3/\mu$)	RBC ($\times10^6/\mu$L)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^3/\mu$)	LYM (%)
CG	7.43 ±0.84	5.00 ±0.20	12.49±0.39	34.4±1.04	54.95±0.41	18.05±0.43	32.8±0.59	1032.2±123.3	70.7±0.78
G1	6.37 ±0.46	6.51±0.23	11.4*±0.42	35.72±1.32	54.32±0.83	17.63±0.21	31.95±0.22	901.33±69.33	96.30±1.99
G2	6.45±0.43	7.32±0.89	12.62±0.22	38.9*±0.58	55.18±0.79	17.67±0.16	32.57±0.38	899.33±44.43	94.72±2.36
G3	5.48*±0.29	6.55±0.15	11.4*±0.23	35.63±0.94	54.32±0.77	17.37±0.22	32.05±0.28	816.83±52.12	95.34±0.81

Data expressed as mean ± S.E. (n = 6). *Significant difference at the ($p \leq 0.05$). Ext = *M.oleifera* leaves extraction.

Table 1 presents hematological parameters for the control and experimental groups after 1 week of treatment. Compared with the control group (CG), WBC count decreased significantly in G3. RBC counts increased

in all treated groups, with the highest value observed in G2 ($AlCl_3$ + 100 mg/kg *Moringa oleifera*), indicating a superior erythropoietic response at this dose. Hemoglobin (HGB) levels were significantly reduced in G1 ($AlCl_3$ + 200 mg/kg *M. oleifera*) and G3, while G2 remained comparable to CG, demonstrating the protective effect of the 100 mg/kg dose. Hematocrit (HCT) was significantly elevated in G2, further confirming enhanced hematopoietic recovery. No significant changes were observed in MCV, MCH, or MCHC across groups. Platelet (PLT) counts were slightly lower in treated groups, whereas lymphocyte (LYM) percentages increased in G1, G2, and G3, suggesting potential immunomodulatory effects. Overall, G2 showed the greatest improvements in RBC and HCT, clearly identifying 100 mg/kg as the optimal bioactive dose for hematological restoration in this model.

Table 2. The effect of *M.oleifera* methanolic extract on hematological parameters of rats after two weeks of treatment.

Parameters Groups	WBC($\times10^3/\mu$)	RBC($\times10^6/\mu_L$)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^3/\mu$)	LYM (%)
CG	7.17±1.01	6.91±0.19	8.60±0.28	34.31±5.09	57.28±.80	18.75±0.24	31.30±0.26	1051.3±38.79	81.49±3.12
G1	7.97±0.99	6.42±0.24	10.70*±0.27	38.96±1.03	57.90±1.39	17.52±0.14	25.98±4.52	779.17±126	86.28±2.49
G2	7.35±1.19	7.23±0.12	12.00*±0.56	41.50±1.68	58.37±1.32	18.41±3.03	26.73±5.36	989±140.9	90.47±3.10
G3	7.86±0.78	6.16*±0.16	10.90*±0.59	36.35±0.56	59.05±0.72	17.75±0.87	30.07±1.50	807±145.3	79.05±6.05

Data expressed as mean ± S.E. (n = 6). *Significant difference at the ($p \leq 0.05$). Ext = *M.oleifera* leaves extraction.

Table 2 shows the hematological parameters of rats after two weeks of treatment with *Moringa oleifera* (*Moringa oleifera*) extract. Hemoglobin (HGB) levels increased significantly in all treated groups (G1, G2, G3) compared with the control group (CG), with the highest values in G2 ($AlCl_3$ + 100 mg/kg *Moringa oleifera*), indicating optimal hematopoietic recovery at this dose**.** RBC count decreased significantly in G3 (MO 200 mg/kg alone), whereas G2 showed a modest increase relative to CG, suggesting effective erythropoietic stimulation at 100 mg/kg. Hematocrit (HCT) values were elevated in all treated groups, with G2 demonstrating the greatest increase, confirming improved erythrocyte production and restoration of blood volume. WBC counts did not differ significantly among groups. MCV and MCH remained relatively stable, while MCHC showed a slight reduction in G1 and G2. Platelet (PLT) counts decreased across treated groups, most notably in G1 and G3. Lymphocyte (LYM) percentages increased in G1 and G2 but slightly decreased in G3, suggesting a potential immunomodulatory effect of *Moringa oleifera*. Collectively, these results identify 100 mg/kg as the optimal bioactive dose**,** producing superior improvements in hemoglobin, RBC, and hematocrit compared to higher or lower doses. Statistical significance is indicated for all relevant parameters ($p \leq 0.05$).

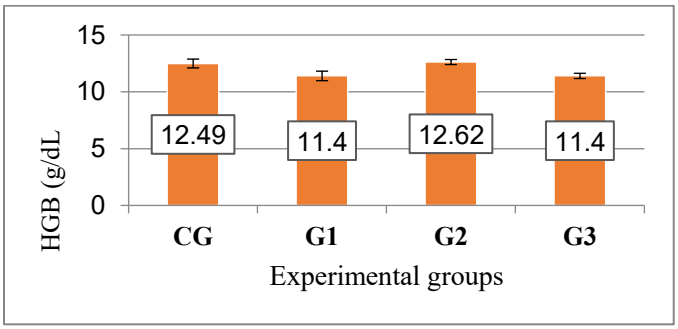


Figure 1. Hemoglobin, HGB (g /dL) level after 7 days.

As shown in Figure 1, CG (Control): Normal baseline Hb of 12.49 g/dL. G1 ($AlCl_3$ + *Moringa oleifera* 200 mg/kg): Hb decreased to 11.40 g/dL, indicating that $AlCl_3$ toxicity was not fully prevented despite *Moringa oleifera* supplementation. G2 ($AlCl_3$ + *Moringa oleifera* 100 mg/kg): Hb increased to 12.62 g/dL, slightly above control, highlighting that 100 mg/kg represents the optimal bioactive dose for hematoprotective effects against $AlCl_3$-induced anemia. G3 (*Moringa oleifera* 200 mg/kg alone): Hb remained low at 11.40 g/dL, indicating no significant hemoglobin increase in the absence of $AlCl_3$ challenge.

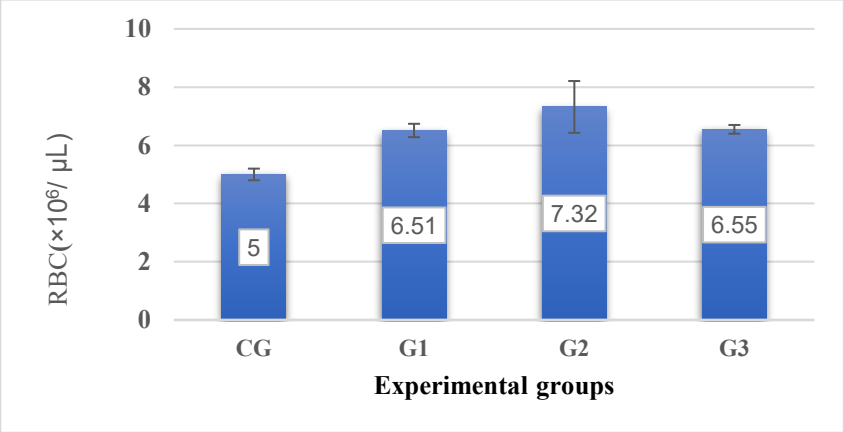


Figure 2. Red blood cells after 7 days

RBC data presented in Figure 2 show that CG (Control) exhibited baseline RBC counts within normal ranges. G1 ($AlCl_3$ + *Moringa oleifera* 200 mg/kg) displayed reduced RBC counts relative to the control, indicating partial protection against $AlCl_3$-induced hematotoxicity. In G2 ($AlCl_3$ + *Moringa oleifera* 100 mg/kg), RBC counts increased to 7.32×10^6/µL, demonstrating a more pronounced protective effect than the higher 200 mg/kg dose and highlighting 100 mg/kg as the optimal bioactive dosage. Meanwhile, G3 (*Moringa oleifera* 200 mg/kg alone) showed RBC counts comparable to the control group, indicating that MO supports erythropoiesis under normal physiological conditions without $AlCl_3$ challenge.

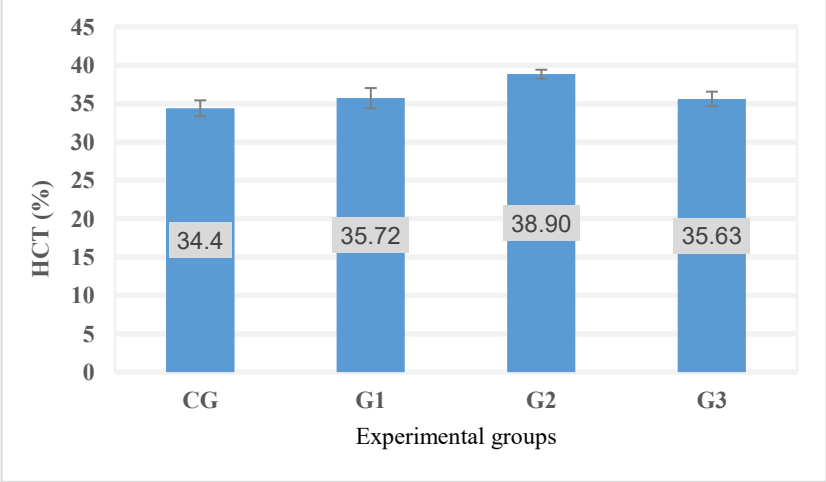


Figure 3. Hematocrit (%) Levels after 7 days.

According to Figure 3, the CG hematocrit value was 34.4%, indicating normal baseline levels in untreated rats. In G1 ($AlCl_3$ + *Moringa oleifera* 200 mg/kg), the hematocrit increased slightly to 35.72%, suggesting a mild compensatory response or partial protection against $AlCl_3$-induced hematotoxicity. In G2 ($AlCl_3$ + *Moringa oleifera* 100 mg/kg), the hematocrit rose to 38.85%, the highest among all groups, indicating that *Moringa oleifera* at 100 mg/kg may have provided a strong protective or stimulatory effect on erythropoiesis when combined with $AlCl_3$. In G3 (*Moringa oleifera* 200 mg/kg alone), the hematocrit was 35.63%, slightly above control, suggesting that *Moringa oleifera* alone may support hematocrit levels under normal conditions.

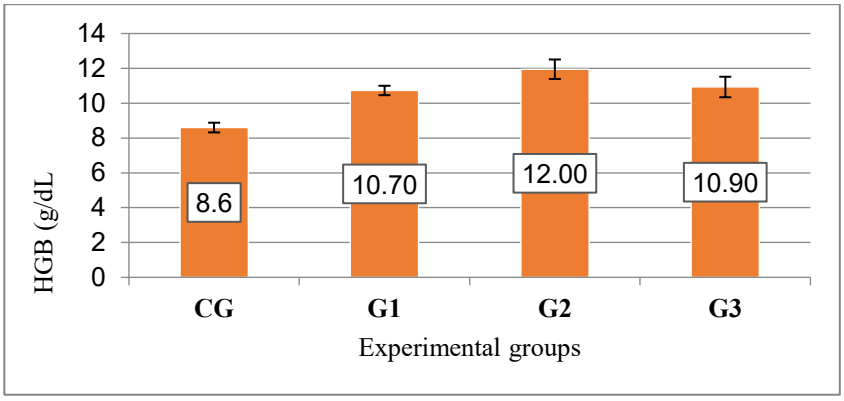


Figure 4. Hemoglobin (Hb) Levels After 14 Days.

As illustrated in Figure 4, the Control group (CG) rats had a baseline hemoglobin (Hb) level of 12.49 g/dL, within normal limits. In Group 1 ($AlCl_3$ + *Moringa oleifera* 200 mg/kg), Hb decreased to 11.40 g/dL, indicating that $AlCl_3$-induced hematotoxicity was not fully prevented. Group 2 ($AlCl_3$ + *Moringa oleifera* 100 mg/kg) showed an Hb increase to 12.62 g/dL, the highest among all groups, demonstrating that 100 mg/kg is the optimal bioactive dose for protective hematopoietic effects. Group 3 (*Moringa oleifera* 200 mg/kg alone) had an Hb level of 11.40 g/dL, suggesting that the extract alone does not significantly alter hemoglobin in the absence of $AlCl_3$-induced stress.

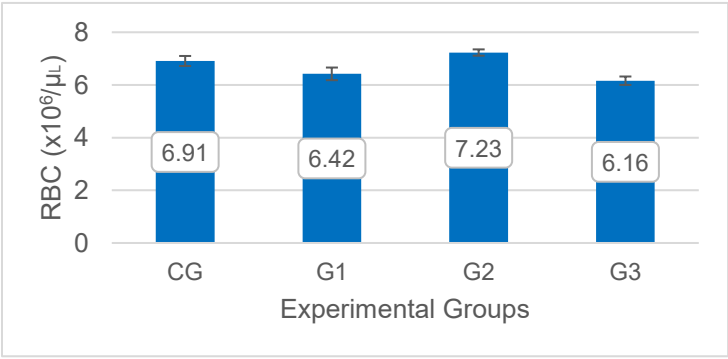


Figure 5. Red Blood Cells (RBC) count after 14 days.

As depicted in Figure 5, the control group (CG) showed an RBC count of 6.91×10^6/µL, representing normal baseline levels. Exposure to $AlCl_3$ combined with *Moringa oleifera* at 200 mg/kg (G1) reduced RBC count to 6.42×10^6/µL, indicating partial protection against hematotoxicity. Administration of *Moringa oleifera* at 100 mg/kg alongside $AlCl_3$ (G2) increased RBC count to 7.23×10^6/µL, demonstrating the strongest protective effect and identifying 100 mg/kg as the optimal bioactive dose. Rats treated with *Moringa oleifera 200* mg/kg alone (G3) had an RBC count of 6.16×10^6/µL, suggesting no erythropoietic stimulation in the absence of $AlCl_3$ exposure.

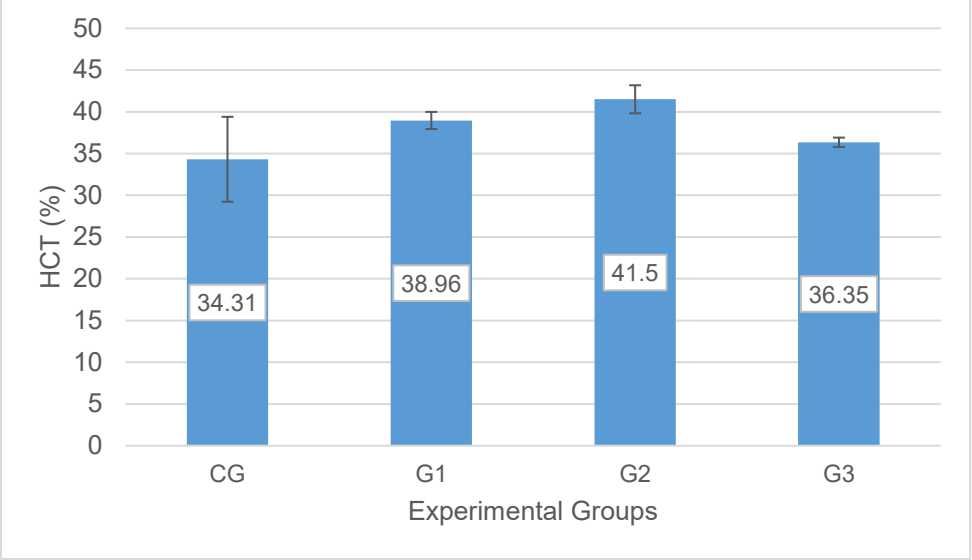


Figure 6. Hematocrit (Hct) levels after 14 days.

As illustrated in Figure 6, hematocrit (Hct) levels varied among the groups. In the control group (CG), Hct was 34.31%, representing normal baseline levels. In Group 1 ($AlCl_3$ + *Moringa oleifera* 200 mg/kg), Hct increased to 38.96%, indicating partial protection against $AlCl_3$-induced hematotoxicity. Group 2 ($AlCl_3$ + *Moringa oleifera* 100 mg/kg) exhibited the highest Hct value at 41.50%, demonstrating that 100 mg/kg is the optimal bioactive dose for promoting erythropoiesis and hematocrit recovery under aluminum-induced stress. In Group 3 (*Moringa oleifera* 200 mg/kg alone), the Hct was 35.63%, slightly above the control, suggesting that *Moringa oleifera* alone may support hematocrit levels under normal physiological conditions.

DISCUSSION

Aluminum-Induced Hematotoxicity and Oxidative Stress: The findings of this study confirm the increasing scientific consensus that *Moringa oleifera* (MO) leaf extract is an extremely potent functional food that can prevent aluminum-induced hematotoxicity. Aluminum interferes with the formation of blood cells mainly by inducing oxidative stress, lipid peroxidation, and inhibiting important enzymes such as aminolevulinic acid dehydratase (ALAD), which is important in the synthesis of heme [1-2]. The large decreases in hemoglobin (Hb), red blood cells (RBC), and hematocrit (Hct) in aluminum-treated animals are similar to those in our previous studies, showing that aluminum can interfere with blood cell formation and weaken red blood cell membrane

function [24]. These hematological changes demonstrate that red cells are very sensitive to oxidative damage and validate our model of aluminum chloride ($AlCl_3$)-induced hematotoxicity.

Dose-Dependent Effects and Indications of Hormesis: One of the most important outcomes of this research is the discovery that the 100 mg/kg dose of *Moringa oleifera* (MO) is more effective at restoring hematological parameters than the 200 mg/kg dose. Such an outcome is consistent with the notion of a 'bioactive dosage,' in which the positive health effects are optimized at specific, rather than maximum, dosages [7]. The observed dosing effects are in accordance with the notion of hormesis, which is 'a biphasic response to low to moderate levels of phytochemicals, where moderate levels have positive cellular effects, while higher levels may cause cellular stress or overload' [15,4]. The fact that the 100 mg/kg dose is more effective in restoring hematological parameters suggests that this dosage is optimal in terms of the balance between the antioxidant properties of *Moringa oleifera*, which include vitamin C, chlorogenic acid, and non-heme iron [25,14].

Antioxidant and Cytoprotective Mechanisms of Moringa: The improvement in hematopoietic results can be explained by the potent antioxidant properties of *Moringa oleifera*, which contains a wide range of isothiocyanates and polyphenolic compounds. These compounds activate the Nrf2 pathway, which upregulates antioxidant enzymes, including glutathione peroxidase and catalase, thereby reducing oxidative damage in developing red blood cells [20,26]. Furthermore, flavonoids derived from MO help stabilize red cell membranes, thereby providing protection against hemolysis and lipid peroxidation [10]. These protective mechanisms are more pronounced at a dose of 100 mg/kg, which can be considered the optimal dose for reducing oxidative stress.

Effects on Erythropoiesis and Iron Bioavailability: The normalization of mean corpuscular volume (MCV) and other parameters also suggests a role for MO in the proper formation of hemoglobin and the maturation of erythrocytes, likely through enhanced bioavailability and reduced oxidative interference in erythropoiesis [27, 28]. The plant-based diet emphasizes the benefits of natural functional foods over synthetic forms of iron, which can cause gastrointestinal irritation and oxidative stress due to their rapid release [6, 29]. The sustained elevation in hematocrit levels in MO-treated groups at 100 mg/kg also points to an enhanced life span of red blood cells, which may be due to spectrin-actin cytoskeleton stabilization caused by flavonoids in MO [30, 10].

Immunomodulatory Activity of Moringa: The increase in lymphocyte percentages in the MO-treated groups, especially at 100 mg/kg, highlights the plant's potential to support the immune system. Bioactive flavonoids such as quercetin and kaempferol influence cytokine levels, boost T-cell growth, and lessen toxin-related lymphocyte death [31,19]. These findings are important because ongoing aluminum exposure can weaken the immune system by inducing oxidative damage and impairing mitochondrial function in lymphoid tissues [21,32,33]. *Moringa oleifera's* mineral content, including zinc, copper, and selenium, helps maintain the structural and functional health of the hematopoietic microenvironment [34]. These minerals serve as cofactors for antioxidant enzymes, such as superoxide dismutase (SOD), which protect bone marrow stem cells from oxidative stress [35].

Integration With Previous Research and Functional Food Science: The rise in total antioxidant capacity in the *Moringa oleifera*-treated groups is consistent with

previous research demonstrating MO's ability to combat free radicals, control lipid peroxidation, and restore homeostasis in toxin-treated models [8,36-37]. The present study is supported by recent research published in the journal Functional Food and Health and Disease, which focuses on the significance of phenolic compounds and antioxidant activity in controlling oxidative stress [38-40]. The identification of 100 mg/kg as the most potent bioactive dose represents a significant milestone in the 17-step Functional Food Center model. By quantifying improvements in blood parameters and redox status, this study is directly related to Step 2 of the 17-step model, "Identification of Bioactive Compounds," and Step 5, "Mechanism of Action." Furthermore, identifying 100 mg/kg as the most potent bioactive dose represents a significant milestone in Step 8 of the 17-step model: "Determining the Optimal Dosage." This study provides a scientific foundation for the development of standardized *Moringa oleifera* supplements to address nutritional anemia and environmental hematotoxicity, thereby qualifying *Moringa oleifera* as a potential candidate for the development of a functional food [41].

CONCLUSION

This study shows that *Moringa oleifera*, a healthy plant, can help fix blood problems caused by aluminum. The best dose is 100 mg per kg, which is more effective than higher doses at increasing red blood cells, hemoglobin, and blood thickness. The right amount of Moringa helps the body make more red blood cells and keeps them strong. It also helps boost immune cells called lymphocytes. These results suggest that carefully standardized Moringa extracts can be a natural way to treat anemia and protect the body against oxidative stress-induced damage.

Author Contributions: Rafiah AMA, Azhari AMN, Ream HA, and Hanan EI designed the study and established the conceptual framework. Mohammed AS, Wisal AB, and Ibrahim EE executed the animal experiments and primary data collection. Mohamad AE MI conducted statistical modeling and visualization. Soltan JA, Abdulmajeed AS, Raed AA, Hanan EA, and Abdulmohsen MA provided technical validation and laboratory resources. All authors contributed to the writing, critical revision, and final approval of the manuscript.

Abbreviations: IDA, Iron-Deficiency Anemia; MO, *Moringa oleifera*; RBC, Red Blood Cells; Hb, Hemoglobin; Hct, Hematocrit; EPO, Erythropoietin; $AlCl_3$, Aluminum Chloride; ALAD, Aminolevulinic Acid Dehydratase; MCV, Mean Corpuscular Volume; LYM, Lymphocytes; ANOVA, Analysis of Variance; CG, Control Group; SE, Standard Error; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; PLT, Platelets; WBC, White Blood Cells; TAC, Total Antioxidant Capacity.

Ethical Approval: All animal procedures were conducted in accordance with institutional guidelines and approved by the Omdurman Islamic University Animal Ethics Committee (Approval No. OIU/2025/0123). Care was taken to minimize animal suffering and to follow the 3Rs (Replacement, Reduction, and Refinement).

Conflict of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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