JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34553

RESEARCH ARTICLE

Evaluating the Effect of *Moringa concanensis* on Aluminium Chloride-induced Anemia in Wistar Rats

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Abstract

Anemia, a widespread global health challenge, can be induced through exposure to deleterious substances such as aluminum chloride. The present investigation explores the potential ameliorative effects of *Moringa concanensis* - a plant acknowledged for its myriad medicinal virtues-against anemia induced by aluminium chloride. The study objective was to scrutinize the impact of *Moringa concanensis* on aluminium chloride-induced anemia in Wistar rats. In the experimental design, thirty Wistar rats were randomly distributed into five distinct groups: a normal control group (untreated), a diseased control group (administered with aluminium chloride at a dose of 0.5 mg/kg body weight), a standard group (treated with Ferrous ascorbate at 30 mg/kg body weight), and two groups receiving low and high doses of *Moringa concanensis* (200 mg/kg and 400 mg/kg body weight, respectively). All groups, with the exception of the normal control, were exposed to aluminium chloride at a dosage of 0.5 mg/kg body weight over a span of 14 days. Hematological indicators were evaluated following standard methodologies, serum ferritin levels were assessed through Electrochemiluminescence immunoassay (ECLIA), and vitamin B12 concentration was quantified using atomic absorption spectroscopy. Furthermore, histopathological alterations were identified through Hematoxylin and Eosin staining procedures. Statistical data were interpreted through one-way ANOVA, succeeded by Tukey's post hoc analysis, considering a p-value below 0.05 as statistically significant. Upon 21 days of continuous treatment with *Moringa concanensis*, both low and high-dose groups exhibited elevation in hematological parameters, serum ferritin, total iron-binding capacity, and vitamin B12 in comparison to the diseased control group. Noteworthy findings were observed in the high-dose group (400 mg/kg body weight), displaying significant improvement compared to the diseased control group ($P<0.001$). Remarkably, the high-dose regimen restored hematological parameters to baseline levels and mirrored the efficacy observed with the standard drug (Ferrous ascorbate). These empirical findings underscore the potential of *Moringa concanensis* as a promising therapeutic candidate for the alleviation of aluminium chloride-induced anemia. These results pave the way for future research endeavors to unravel the precise mechanisms driving these protective effects.

Keywords: Anemia, Aluminium Chloride, *Moringa concanensis*, Protective Effect

1. Introduction

Anemia, characterized by reduced hemoglobin (Hb) levels in the blood, is a symptom of various underlying conditions. These range from increased hemolysis and deficiencies in essential nutrients like folic acid to a mix of genetic and environmental factors[1]. The normal fluctuation of Hb levels due to age, gender, pregnancy status, and other determinants is well-documented. For instance, elevated Hb levels at birth decrease within the first few months, increase during childhood, stabilize in adulthood and decline in later life[2].

Globally, anemia affects about one-third of the population, or approximately 5.5 billion people,

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Article Received on: 25.09.2023 Revised on: 30.09.2023 Accepted on: 05.12.2023

with varying prevalence rates across different demographic groups. Children aged 0-12 years exhibit a 40% prevalence, women 35%, and men 18%4. This variation underscores the multifaceted nature of anemia and its causes. Iron deficiency, thalassemia, hemoglobinopathies, folate deficiencies, and parasitic infections like malaria and hookworm are major contributors. Additionally, Vitamin B12 deficiency and bone marrow failure diseases also play a significant role[3].

To better understand the global impact of anemia, it's essential to consider its significant role in increasing risks of maternal and child mortality, particularly in developing countries. These regions often face challenges in diagnosing and managing anemia, further complicating its global burden. The WHO's definition of anemia, based on Hb level thresholds, is a critical tool for measuring and addressing this public health issue globally[4].

Medicinal plants have historically been vital in treating various ailments. The World Health Organization recognizes a medicinal plant as one containing bioactive compounds beneficial for therapy or drug synthesis[5,6]. *Moringa concanensis*, a member of the *Moringaceae* family, is indigenous to Tamil Nadu, India, and exhibits potential against diseases like cancer, epilepsy, Parkinson's disease, and diabetes[7-10]. However, its effectiveness against anemia is yet to be established.

Aluminum chloride ($AlCl_3$) is known to induce anemia by interfering with several bodily functions. It affects iron metabolism, leading to iron deficiency, a significant cause of anemia. $AlCl_3$ also induces oxidative stress, which can damage red blood cells and impair their function. Furthermore, $AlCl_3$'s impact on the bone marrow can inhibit the production of red blood cells. This research seeks to explore the potential of *Moringa concanensis* in treating $AlCl_{3f}$-induced anemia, an area that has not been extensively studied yet.

2. Materials and Methods

Experimental procedures were executed within the Pharmacology Department of KLE College of Pharmacy, Bengaluru, India. Animals were housed per CPCSEA guidelines in an Animal Facility.

3. Preparation of Plant Material

Leaves of *Moringa concanensis* have been collected from the Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bangalore. Dr. P. E. Rajasekharan, Principal Scientist and Nodal Officer GAC, Division of Plant Genetics Resources, has authenticated the plant. The leaves were rinsed with purified water and dried in shade for 10 days, and coarse powder was prepared and made to pass sieve number 40.

4. Plant Extraction

A previously documented method[11] was employed for the extraction process using approximately 500 grams of the powdered, dried plant. This material was placed into a Soxhlet apparatus (supplied by Borosil, India) and extracted with ethanol as the solvent. Post extraction, the ethanol-based extract was filtered to remove impurities. This filtrate was then subjected to evaporation under diminished pressure, at a temperature maintained below or equal to 50°C, utilizing a vacuum evaporator (manufactured by Perfit, India). This procedure yielded a crude extract, which was subsequently stored in desiccators for preservation. The leftover plant residue post-extraction was discarded. The crude extract's weight was documented upon collection. This crude extract was diluted in distilled water for subsequent analyses or applications to establish final concentrations of 200 and 400 mg/kg b.w.

5. Animal and Experimental Design

30 male Albino rats, an average weight of 265g obtained from Vaarunya Biolabs Private Limited, maintained under the CPCSEA guidelines in the animal facility at KLE College of Pharmacy, fed with the standard diet. The diet was commercial feed purchased from VRK Nutritional Solutions, Pune, Maharashtra, and water was given *ad libitum*.

The animals were grouped randomly into five groups[12]:

- Normal Control (NC): No treatment is given,
- Diseased Control (DC): Animals received the 0.5 mg/kg b.w. $AlCl_3$ for 14 days, and free access to feed and water,

- Standard group (STD): Animals received $AlCl_3$ 0.5 mg/kg b.w. for 14 days after that standard treatment of Ferrous ascorbate 30 mg/kg b.w. was administered,
- Low Dose (LD): Animals received $AlCl_3$ 0.5 mg/kg b.w. for 14 days; after that, a dose of 200 mg/kg b.w. of EEMCL was given for 21 days,
- High Dose (HD): Animals first received $AlCl_3$ 0.5 mg/kg b.w. for 14 days. After that, a dose of 400 mg/kg b.w. of EEMCL was given for 21 days.

6. Evaluation of Hematological Parameters

After completion of the experiment, approximately 5 to 6 ml of blood was collected by hematocrit capillary tube from the retro-orbital puncture of each rat. The 2 ml of blood samples were mixed with one to two drops of EDTA solution and immediately utilized to assess various blood metrics. Haemoglobin (Hb): estimated using a hemoglobin kit in a Semi-autoanalyser (Robonik-Prietest biochemistry analyzer). WBCs, RBCs, and platelets were counted by Neubauer's chamber (Uniglobal Business Products, Greater Noida). Packed Cell Volume (PCV): estimated in the centrifuge, 1000 rpm for 15 min (Remi R8C Plus-Laboratory Centrifuge). Mean Cell Volume (MCV): calculated using a formula, MCV = (PCV×10) ÷ RBCs (10^{-12}). Mean Cell Hemoglobin (MCH): calculated using a formula, MCH = (Hb×10) ÷ RBCs (pg). Mean Cell Hemoglobin Concentration (MCHC): calculated by the formula, MCHC = (Hb×100) ÷ PCV (g/dL)[12-15].

1 ml blood sample was taken in an empty tube without EDTA and left for 30 min for coagulation. A blood sample was centrifuged at 1000 rpm for 15 min, and the serum was collected to measure SGPT and SGOT. SGPT and SGOT were estimated using a previously published Prietest Touch Plus Biochemistry Analyzer method[15].

7. Evaluation of Serum Ferritin, Total Iron Binding Capacity (TIBC), and Vitamin B12 (Vit. B12)

An Electrochemiluminescence immunoassay (ECLIA) was used with the Elecsys ferritin kit from (Roche Diagnostics International Ltd.) to determine serum ferritin levels in human serum[16]. To evaluate the Total Iron Binding Capacity (TIBC), serum was treated with an excess amount of ferric iron to fully saturate siderophyllin. Following this, any remaining free iron was eliminated using magnesium carbonate powder, as per Ramsay's method[17]. Atomic absorption spectroscopy, described by Karmi[18], determines Vitamin B12 levels.

8. Histological Examination of Liver

Liver histological analyses were conducted following established standard operating procedures. A liver specimen was carefully dissected, and a segment of the liver tissue was subsequently fixed in 10% formalin solution, followed by embedding within paraffin. Serial tissue sections, each with a thickness of 5 µm, were obtained using a rotary microtome and then subjected to staining with H and E in compliance with routine pathological laboratory protocols[19].

9. Statistical Analysis

Data were statistically analyzed using One-way ANOVA, followed by Tukey's post hoc test, with GraphPad Prism software (version 8.0). Significance was established at a p-value of <0.05. Results are presented as Mean ± S.E.M. Symbols $^{\#}P<0.05$ and $^{\#\#}P<0.01$ represent significant deviations from normal values. Conversely, $^{*}P<0.05$ and $^{**}P<0.01$ indicate significant differences compared to the control group.

10. Results

10.1 Effect of Ethanolic Extract *Moringa concanensis* Leaves (EEMCL) on Hematology

The results for hematology are shown in Table 1 Hb, RBCs count, WBCs count, and platelet count (Figure 1). In group DC, data shows significantly lower haemoglobin, WBCs, RBCs, and platelet counts. The group is solely treated with $AlCl_3$ for 14 days. However, the above-mentioned parameters have been significantly improved upon the treatment with EEMCL in LD (200mg/kg) and HD (400mg/kg) groups. Especially in the HD group shows improved hematology, equivalent to the normal animals (P <0.001).

Table 2 shows the blood indices (PCV, MCV, MCH, and MCHC). The PCV is significantly lower in the DC compared to NC animals, which indicates the relatively lower volume of blood cells in a unit of blood (Figure 2). But PCV is restored to normal animals in HD group animals ($P<0.05$). The MCV in the DC group is higher than the normal animals, but in the LD and HD groups, it has been stabilized in HD animals. Similarly, MCH and MCHC are slightly higher in the DC group but get stabilized in LD and HD groups ($P <0.001$).

10.2 Effect of Ethanolic Extract *Moringa concanensis* Leaves on Serum Ferritin, Total Iron Binding Capacity, Vitamin B12, and Liver Biomarkers

Table 3 shows the values for biochemical parameters like SGOT and SGPT, serum ferritin, TIBC, and vitamin B12. The low dose and high dose of EEMCL effectively lower SGOT and SGPT in groups LD and HD (Figure 3). But a high dose of EEMCL lowers the SGOT and SGPT ($P<0.001$). In terms of

Table 1. Effects of EEMCL on hematological parameters in $AlCl_3$-induced anemia in rats

Sl. No.	Groups	Hb (g/dL)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Platelets (10^3/mm^3)
1.	NC	14.4±0.1713	8.232±0.1561	7.185±0.1943	775.5±27.34
2.	DC	8.050±0.1839$^{###}$	3.745±0.1117$^{###}$	3.618±0.2233$^{###}$	399.5±6.965$^{###}$
3.	STD	13.70±0.1155***	7.955±0.0513***	6.637±0.0931***	625.3±26.66***
4.	LD	11.97±0.1783***	6.612±0.2350***	6.040±0.2074***	566.2±18.71***
5.	HD	14.43±0.1430***	8.655±0.2348***	7.888±0.1099***	850.2±17.78***

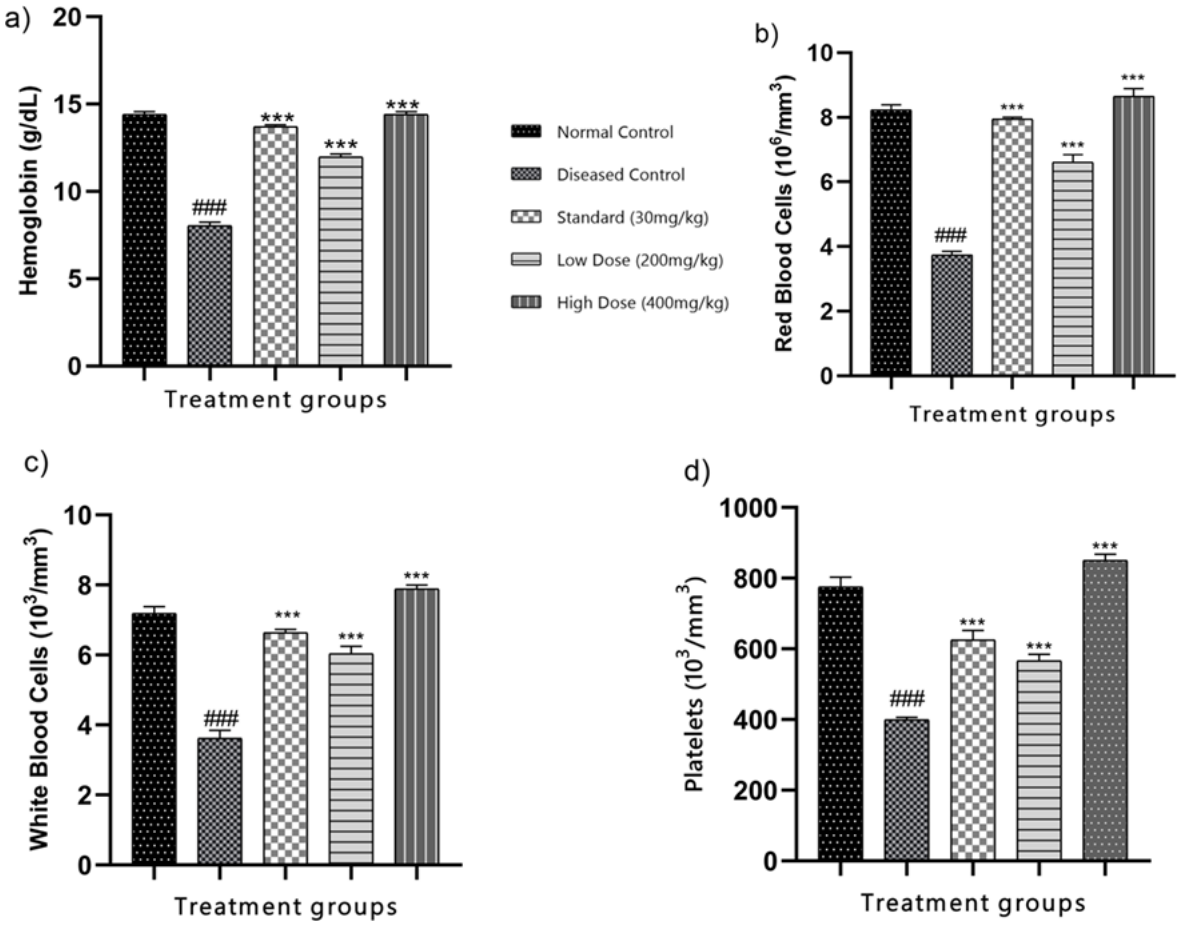

Figure 1. EEMCL treatment effects on $AlCl_3$-induced anemia in rats: **(a).** Hb, **(b).** RBC, **(c).** WBCs, **(d).** Platelets. Data: Mean ± SEM (n=6). $^{###}P<0.001$ vs. normal; $^{***}P<0.001$ vs. diseased control.

Table 2. Effects of EEMCL on blood indices in $AlCl_3$ induced anemia in rats

Sl. No.	Groups	PCV (%)	MCV (10^{-12})	MCH (pg)	MCHC (g/dL)
1.	NC	42.88±0.678	53.04±1.467	17.53±0.464	33.59±0.387
2.	DC	24.27±1.158$^{###}$	64.85±2.603$^{##}$	21.61±0.954$^{###}$	36.57±0.601
3.	STD	37.88±0.659***	48.53±0.734***	17.44±0.228***	31.71±1.033**
4.	LD	38.40±0.848***	59.44±2.435	18.29±0.613**	31.19±0.810***
5.	HD	43.37±0.737***	50.32±1.212***	16.76±0.458***	33.34±0.787*

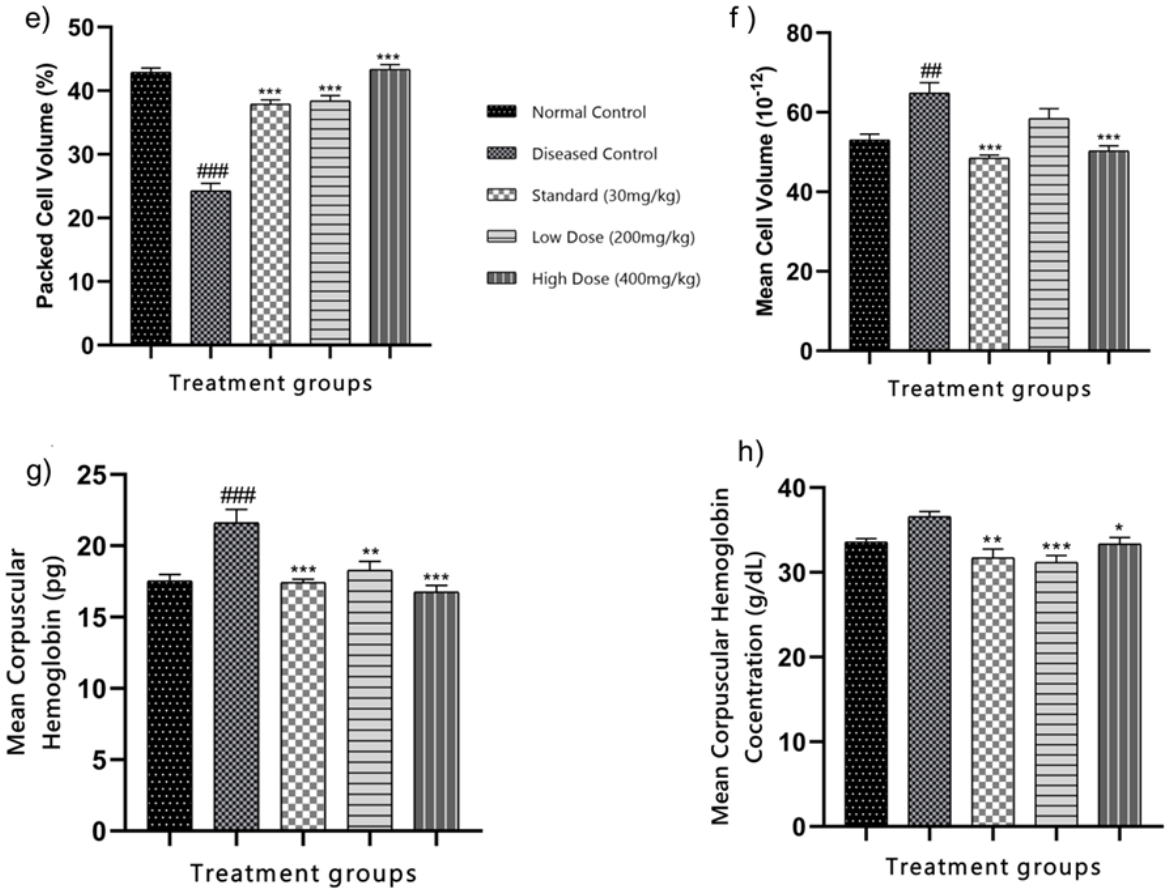

Figure 2. EEMCL treatment impact on AlCl3-induced anemia in rats: **(e).** PCV, **(f).** MCV, **(g).** MCH, **(h).** MCHC. Data: Mean ± SEM (n=6). $^{###}$P<0.001, $^{##}$P<0.01 vs. normal; ***P<0.001, **P<0.01, *P<0.05 vs. diseased control.

serum ferritin, a high dose is much more effective in restoring the serum ferritin to normal levels (P<0.001).

10.3 Histopathological Evaluation of $AlCl_3$-induced Anemia

Histopathological report of $AlCl_3$-induced anemia revealed vascular degeneration with little necrosis, the periportal region shows moderate inflammatory cell infiltration compared to control group which represented the usual hepatic cellular organization.

The perivenular, periportal and mid zonal hepatocytes seemed to be intact and also a refined degenerative interchange and less cellular necrosis were visible in Standard treated group with $AlCl_3$ and EEMCL treated groups of doses 200 mg/kg and 400 mg/kg with respect to the control groups (Figure 4).

11. Discussion

In this research, we have examined the preventive impact of the ethanol-derived extract from *Moringa*

Table 3. Effects of EEMCL on SGOT and SGPT, serum ferritin, TIBC, and Vitamin B12 in $AlCl_3$-induced anemia in rats

Sl. No.	Groups	SGPT (U/L)	SGOT (U/L)	Serum ferritin (µg/dL)	TIBC (µg/dL)	Vitamin B_{12} (pmol/ml)
1.	NC	29.77±1.32	39.38±3.26	598.9±6.062	321.54±2.34	998.4±3.189
2.	DC	73.65±2.19$^{###}$	88.48±2.12$^{###}$	195.9±5.338$^{###}$	299.83±1.89$^{#}$	893.72±2.34$^{#}$
3.	STD	59.91±1.89***	68.76±2.89**	550.7±31.9***	348.91±3.21**	909.34±3.89
4.	LD	48.87±1.07***	55.76±2.31***	420.8±15.43***	324.61±1.72	912.64±3.99
5.	HD	46.49±2.19***	49.54±1.65***	610.0±28.91***	381.53±0.89**	1032.44±2.10*

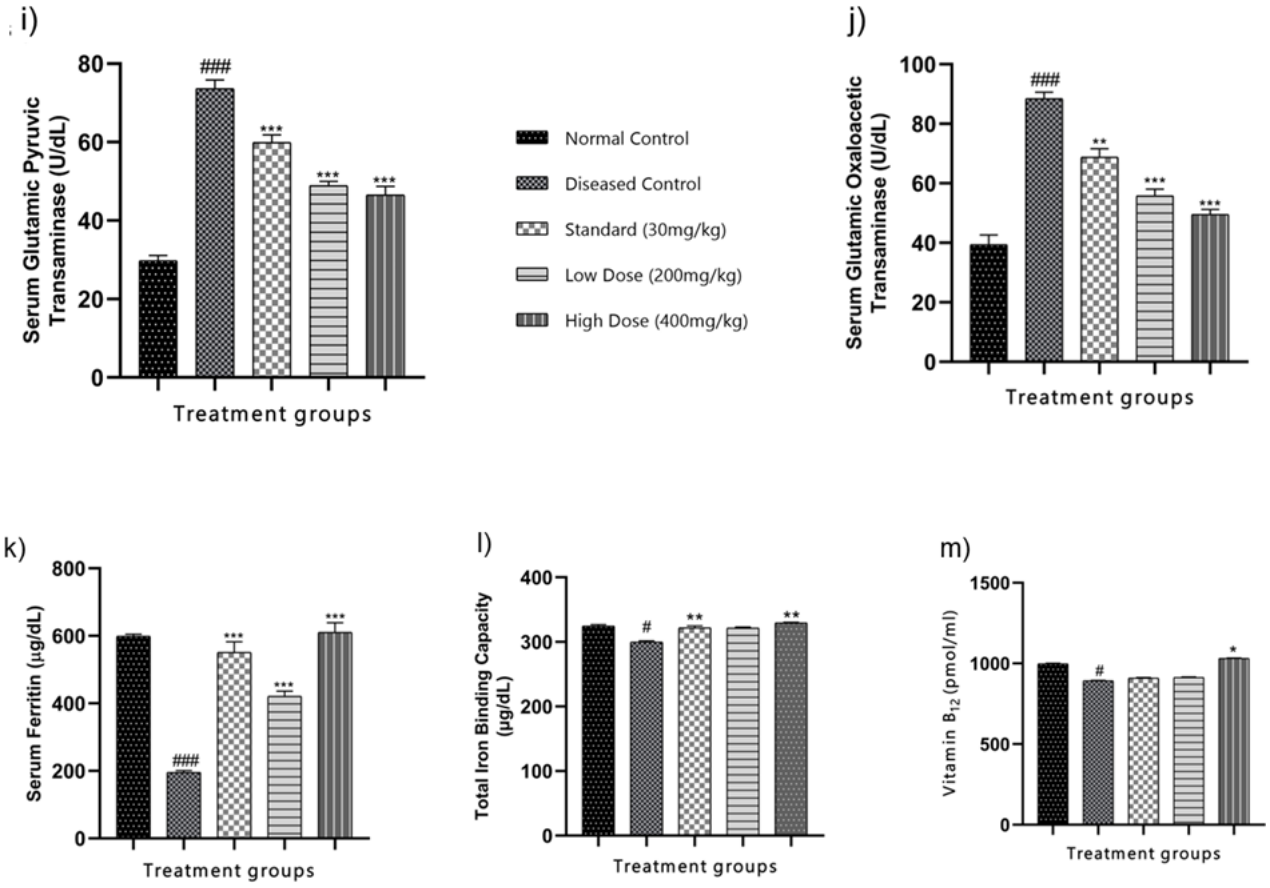

Figure 3. EEMCL treatment impact on $AlCl_3$-induced anemia in rats: **(i).** SGPT, **(j).** SGOT, **(k).** Serum ferritin, **(l).** Total iron-binding capacity, **(m).** Vitamin B12. Data: Mean ± SEM (n=6). ###P<0.001, ##P<0.01, #P<0.05 vs. normal; ***P<0.001, **P<0.01, *P<0.05 vs. diseased control.

concanensis leaves (EEMCL) on aluminium chloride-induced anemia in rats. This study was carried out by taking two different doses of EEMCL: the lower dose of 200 mg per kg b.w. and the higher dose of 400 mg per kg b.w. The exact mechanism of Aluminium chloride in causing the anemia is still unclear, but it is evident that it might be interfering with the production of heme, this either could be due to enzyme activity inhibition or the uptake of iron for hemoglobin synthesis[8]. In a different study, it was found that the cause of aluminum toxicity is due to the binding of this metal with transferrin which also damages the liver.

The potential mechanisms by which *Moringa concanensis* may have ameliorated the anemia include its rich phytochemical profile, known to possess anti-oxidative, anti-inflammatory, and possibly iron-chelating properties. These attributes might help in mitigating the oxidative stress and liver damage caused by aluminium chloride, thereby improving hematopoiesis and iron metabolism. Furthermore,

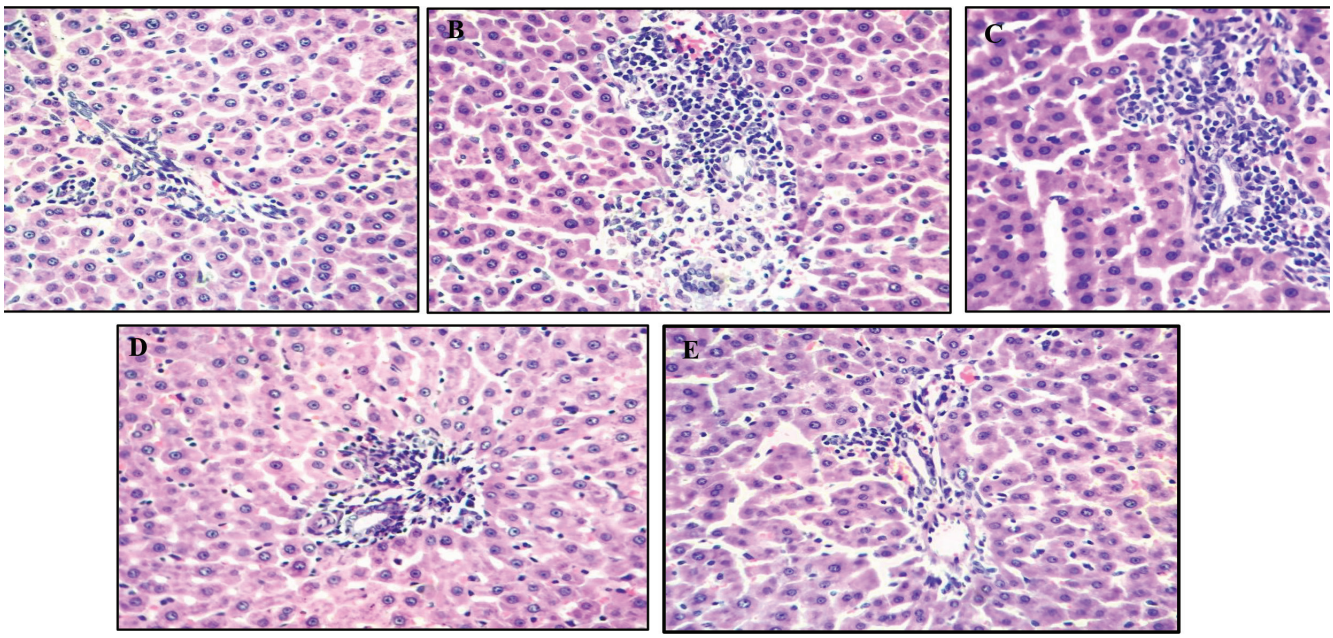

Figure 4. Effect of ethanolic extract *Moringa concanensis* treatment on liver- H and E staining (40×). **(A).** Normal Control, **(B).** disease, **(C).** standard group, **(D)** and **(E).** *Moringa concanensis* (200 mg/kg and 400 mg/kg).

Moringa concanensis could influence erythropoiesis directly or indirectly by modulating various hematopoietic factors.

The current research observed that throughout the testing phase, there was a notable rise in blood cell production and other hematological metrics in the groups treated with the ethanol-derived extract from *Moringa concanensis* leaves. This increase was significant when compared to the diseased control group ($P<0.001$). In the estimation, normal laboratory procedures and calculations were used for the assessment of hematological parameters and blood indices (PCV, MCV, MCH, and MCHC) respectively.

This study found that anti-anemic effect was achieved at the low dose (200 mg/kg body weight) and elevated the hematological parameters when compared to the diseased control group, but this low dose was not as effective as that of the standard drug (Ferrous ascorbate).

The high dose (400 mg/kg body weight) was significantly as effective as the standard drug. So, the best anti-anemic activity effect was provided by the high dose. This dose elevated the hemoglobin, RBCs count, WBCs count, and platelet counts to normal values. As hemoglobin helps to supply oxygen to cells and carry back carbon dioxide as a waste product. An insufficient amount of hemoglobin in the body, could not supply enough oxygen to the cells in body that they require to survive[20].

As the RBC lacks a nucleus, this allows them to store hemoglobin, enabling them to transport more oxygen. WBC on the other hand provides immunity. The low levels of WBCs in an individual would result in more and worse infections (bacterial and viral infections). Neutrophils digest bacteria, and monocytes become macrophages and engulf pathogens. Lymphocytes, composed of B and T cells, offer protection against various pathogens. Platelets are cell fragments circulating in the blood; upon activation, they facilitate blood clotting[21].

The WBCs count and platelet in the high-dose group in little higher than the normal control. This could be due to the effect of the drug and might come down to normal when the effect of the drug wears off. Packed cell volume measures the volume of packed red blood cells relative to whole blood[22]. The measurement of the average size and volume of red blood cells is MCV. It determines the type of anemia as either microcytic or macrocytic[23].

MCH measures the hemoglobin content in each Red Blood Cell (RBC), while MCHC represents the concentration of hemoglobin in a given volume of RBCs[24]. The study revealed that the EEMCL is capable of significantly decreasing the liver biomarkers, SGPT, and SGOT, compared to the diseased control group. The

best effect was seen in the high dose of EEMCL. This research also revealed that the high-dose group exhibited a significant rise in serum ferritin levels compared to the diseased control, matching the levels seen in the normal control. However, regarding total iron binding capacity and vitamin B12, the EEMCL displayed minimal or no impact. For the histopathology, the drug-treated groups show healthier hepatocytes as near equivalence to normal control when compared to diseased control.

The limitations of the current study include the lack of exploration into the detailed molecular pathways through which *Moringa concanensis* exerts its therapeutic effects. Moreover, the study did not assess the long-term implications of EEMCL treatment, including any potential side effects. Future research should focus on elucidating the molecular mechanisms of action, investigating the long-term safety profile of EEMCL, and assessing its impact on other biological systems. Additionally, studies examining levels of transferrin saturation and histopathology of bone marrow are recommended to provide a more comprehensive understanding of the drug's impact.

12. Conclusion

Moringa concanensis leaves confirm the anti-anemic activity by increasing hemoglobin, RBCs, WBCs, and Platelet count. It did not only increase the hematological parameters but also increased serum ferritin levels in anemic rats. Therefore, it could be developed as an herbal source for anemia other than ferrous salts as medicine. Further research is needed regarding the safety and appropriate product formulations to ensure safety.

13. Acknowledgment

The Department of Pharmacology at KLE College of Pharmacy, Bengaluru, is gratefully acknowledged for their invaluable support in providing the necessary resources and assistance that significantly contributed to the conduct and submission of this research article.

14. Ethical Approval

Approval was secured from the Institutional Animal Ethics Committee (IAEC) of KLE College of Pharmacy, Bengaluru, India (626/PO/Re/S/02/CPCSEA).

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