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Hematological effects of lead exposure and Moringa intervention in rats

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Abstract

In this study, the hematological effects of lead acetate and defatted *Moringa oleifera* seed meal (DMOSM) were investigated in male *Wistar* rats. Eighty *Wistar* rats were assigned into 5 groups (16 rats each) as follows: Group I (negative control), II (lead acetate solution at 480 mg/kg), III (lead acetate solution and DMOSM at 480 mg/kg each, simultaneously), IV (lead acetate solution at 480 mg/kg for the first 14 days, followed by DMOSM at 480 mg/kg for the next 14 days, and V (DMOSM at 480mg/kg). Treatments were administered orally and daily for 28 days. Blood was collected on days 7, 14, 21, and 28 and processed for hematological examination. Results revealed non-significant ($p > 0.05$) increases in red blood cell parameters (PCV, Hb, RBC) at 7, 14, 21, and 28 days in groups II, III, IV, and V compared to the negative control (Group I). Group III exhibited the highest values, suggesting a potential compensatory mechanism against lead-induced anemia. White blood cell indices showed non-significant ($p > 0.05$) fluctuations, with Group III displaying a persistent immunomodulatory effect. Neutrophil counts were significantly ($p < 0.05$) higher in all treatment groups at 7 days, indicating a potential early inflammatory response. In conclusion, the results suggest a potential ameliorative effect of DMOSM on lead-induced haematological alterations. The study, therefore, highlights the intricate interplay between lead toxicity and the protective effects of *M. oleifera*, underscoring the need for further research to elucidate the underlying mechanisms and validate these findings in diverse populations.

Keywords: Lead acetate, Defatted *Moringa oleifera* seed meal, Red blood cell, White blood cell

Introduction

Lead toxicity poses a significant threat to public health, with established adverse effects on various physiological systems, particularly hematological parameters (Ara et al., 2015; Mitra et al., 2022; Olufemi et al., 2022). Exposure to lead acetate is known to disrupt the delicate balance of red and white blood cells, leading to conditions such as anemia and immune system dysregulation (Ibrahim et al., 2012; Kianoush et al., 2013; Ilesanmi et al., 2022). Despite the pervasive nature of lead-induced hematological alterations, effective interventions remain elusive, necessitating a comprehensive exploration of potential protective measures.

The multifaceted nature of lead-induced hematological alterations underscores the pressing need for targeted interventions to mitigate the adverse effects on red and white blood cell parameters. Traditional approaches have fallen short in providing comprehensive solutions to this public health challenge. Our study addresses this research gap by focusing on defatted *M. oleifera* seed meal (DMOSM), a natural intervention known for its antioxidant and anti-inflammatory attributes. The potential hepatoprotective properties of *M. oleifera* (Brilhante et al., 2017; Kou et al., 2018; Alia et al., 2022; Jikah & Edo, 2023) make it a promising candidate for addressing lead-induced hematological disruptions, and a thorough investigation of its temporal dynamics and underlying mechanisms is critical for establishing its efficacy.

The intricate interplay between lead toxicity and the protective effects of *M. oleifera* remains relatively unexplored in the context of hematological responses. Previous studies have highlighted the antioxidant and anti-inflammatory potential of *M. oleifera* (Kou et al., 2018; Xu et al., 2019; Abd-Elnaby et al., 2022), but a nuanced understanding of its effects over a specific timeframe is lacking. This study aims to contribute significantly to the existing knowledge by unraveling the temporal dynamics of hematological responses over 28 days. The insights gained from this investigation are expected to inform more targeted and informed interventions against lead-induced toxicity, emphasizing the importance of *M. oleifera* seed meal as a potential natural remedy. Therefore, this study investigated the hematological responses triggered by lead acetate exposure and assessed the potential ameliorative effects of defatted *M. oleifera* seed meal (DMOSM) in a 28-day experimental investigation utilizing *Wistar* rats.

Material and methods

Collection and identification of the plant material

The seeds of *Moringa oleifera* were collected from Ruma, Batsari Local Government Area of Katsina state. They were authenticated by a taxonomist at the Herbarium, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, where a voucher number 571 was deposited.

Extraction of *Moringa oleifera*

The fresh seeds were allowed to dry in a shed at room temperature for two weeks. The dried plant seeds were pulverized to powder using a mortar and pestle. Exactly 522.750 g of the powdered seeds were weighed out and utilized for the mechanical cold press extraction (Wandhekar, 2023).

Identification of phytochemical constituents

The phytochemical constituents of DMOSM were determined using methods described by Evans (2009).

Acute toxicity studies

The acute toxicity studies (LD_{50}) for lead acetate and DMOSM were carried out as described by Chinedu et al. (2013). A dose of 480 mg/kg for both test agents (one-tenth of the highest dose, 4800 mg/kg), was then selected for the study based on the absence of observable signs of toxicity and mortality at the highest administered dose.

Preparation of lead acetate solution and defatted *Moringa oleifera* seed meal

The lead acetate salt (Mayer and Baker®) solution was prepared by dissolving 8 g of salt in 20 mL of deionised water to obtain a 400 mg/mL concentration, while the defatted M. oleifera seed cake was ground to powder using a mortar and pestle and then sieved. Exactly 5 g of the fine powder of the defatted *Moringa oleifera* seed cake was dissolved in 20 mL of distilled water to obtain a 250 mg/mL concentration of the seed meal used for this study.

Experimental design

A 28-day experimental study was conducted using eighty (80) male *Wistar* rats randomly divided into five groups (n=16 per group): Group I served as the negative control, receiving distilled water; Group II received lead acetate solution (480 mg/kg) daily via oral gavage; Group III received lead acetate solution (480 mg/kg) and DMOSM (480 mg/kg) simultaneously; Group IV received lead acetate (480 mg/kg) for the first 14 days and subsequently DMOSM (480 mg/kg) for the next 14

days; Group V received only DMOSM (480 mg/kg). All treatments were administered daily for 28 days.

Hematological assessments

Blood samples were collected at 7, 14, 21, and 28 days for hematological analyses. Parameters including packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured. White blood cell (WBC) counts and differential counts for neutrophils and lymphocytes were also determined.

Data analysis

Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* test to assess significant differences among groups. Results were considered significant at $p \leq 0.05$. Data were presented as mean ± standard error of the mean (SEM).

Ethical considerations

The study was conducted following ethical guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) before the commencement of the experiment. All efforts were made to minimize animal suffering by ethical standards.

Results

The phytochemical constituents present in DMOSM were reducing sugar, alkaloid, saponin, triterpenes, and cardiac glycoside. At 7 days of administration, the PCV, Hb, and RBC were non-significantly ($p > 0.05$) higher in groups II, III, IV, and V than in group I, with the highest in group III (Table 1). The MCV, MCH, and MCHC showed no significant ($p > 0.05$) difference across the groups. There was non-significantly ($p > 0.05$) lower TWBC and lymphocyte count in group III compared to the other groups. The neutrophil count was significantly ($p < 0.05$) higher in groups II, III, IV, and V than in the control (group I), with the highest in group IV (Table 1).

At 14 days of administration, there was non-significantly ($p > 0.05$) higher PCV, Hb, and RBC in groups II, III, IV, and V, compared to group I (Table 2). No significant differences ($p > 0.05$) existed for the MCV, MCH, and MCHC in all the groups. The TWBC, neutrophil, and lymphocyte counts were non-significantly ($p > 0.05$) higher in group IV than in the other groups (Table 2).

At 21 days of administration, the PCV, Hb, and RBC were non-significantly ($p > 0.05$) higher in groups II, III, IV, and V than in group I, with the highest in group V (Table 3). The MCV, MCH,

and MCHC showed no significant ($p > 0.05$) differences in all the groups. There were non-significantly ($p > 0.05$) higher TWBC, neutrophil, and lymphocyte counts in group III than in the other groups (Table 3).

At 28 days of administration, the PCV, Hb, and RBC showed no significant ($p > 0.05$) differences across the groups but were non-significantly lower in group I compared to groups II, III, IV, and V (Table 4). There were no significant ($p > 0.05$) differences across the groups in the MCV, MCH, and MCHC. The TWBC, neutrophil, and lymphocyte counts were non-significantly ($p > 0.05$) higher in group III than in the other groups (Table 4).

Table 1. Hematological parameters (at one week) of *Wistar* rats administered defatted *Moringa oleifera* seed meal and/or lead acetate

	Group				
Parameter	I (Control)	II (Lead acetate only)	III (Lead acetate +*Moringa oleifera*)	IV (Lead acetate followed by *Moringa oleifera*)	V (*Moringa oleifera* only)
Packed cell volume (%)	41.25 ± 3.59	47.50 ± 3.30	53.00 ± 4.81	48.50 ± 2.26	49.50 ± 5.42
Haemoglobin concentration (g/dL)	13.70 ± 1.20	15.80 ± 1.10	17.63 ± 1.60	16.15 ± 0.75	16.48 ± 1.80
Red blood cells ($\times 10^6$/µL)	6.83 ± 0.59	7.90 ± 0.54	8.78 ± 0.80	8.08 ± 0.37	8.23 ± 0.90
MCV mean corpuscular volume (*f*L)	60.44 ± 0.07	60.34 ± 0.14	60.40 ± 0.21	60.06 ± 0.15	60.18 ± 0.24
Mean corpuscular hemoglobin (pg)	20.07 ± 0.04	20.00 ± 0.06	20.08 ± 0.06	20.00 ± 0.05	20.03 ± 0.09
Mean corpuscular hemoglobin concentration (%)	33.21 ± 0.03	33.26 ± 0.03	33.25 ± 0.03	33.30 ± 0.02	33.29 ± 0.03
Total white blood cells ($\times 10^3$/µL)	5.20 ± 0.36	6.70 ± 0.64	4.60 ± 0.70	6.63 ± 0.73	4.55 ± 0.56
Neutrophil ($\times 10^3$/µL)	0.78 ± 0.19[a]	1.18 ± 0.17[ab]	1.38 ± 0.19[ab]	1.80 ± 0.07[b]	1.20 ± 0.16[ab]
Lymphocyte ($\times 10^3$/µL)	3.78 ± 0.70	5.23 ± 0.93	3.23 ± 0.65	4.68 ± 0.59	3.38 ± 0.42

Table 2. Hematological parameters (at two weeks) of *Wistar* rats administered defatted *Moringa oleifera* seed meal and/or lead acetate

	Group				
Parameter	I (Control)	II (Lead acetate only)	III (Lead acetate +*Moringa oleifera*)	IV (Lead acetate followed by *Moringa oleifera*)	V (*Moringa oleifera* only)
Packed cell volume (%)	41.25 ± 1.38	44.50 ± 1.32	43.00 ± 3.44	45.50 ± 0.65	41.75 ± 1.32
Haemoglobin concentation (g/dL)	13.73 ± 0.44	14.83 ± 0.44	14.33 ± 1.13	15.13 ± 0.21	13.90 ± 0.42
Red blood cells ($\times 10^6$/µL)	6.85 ± 0.22	7.50 ± 0.27	7.33 ± 0.55	7.45 ± 0.17	6.90 ± 0.21
MCV mean corpuscular volume (*f*L)	60.21 ± 0.18	59.37 ± 0.32	58.67 ± 0.97	61.12 ± 0.59	60.51 ± 0.31
Mean corpuscular haemoglobin (pg)	20.00 ± 0.06	19.78 ± 0.10	19.52 ± 0.32	20.32 ± 0.19	20.11 ± 0.11
Mean corpuscular haemoglobin concentration (%)	33.28 ± 0.05	33.31 ± 0.02	33.32 ± 0.04	33.24 ± 0.04	33.24 ± 0.04
Total white blood cells ($\times 10^3$/µL)	3.73 ± 0.24	3.68 ± 0.30	4.15 ± 1.32	4.95 ± 1.73	3.18 ± 0.68
Neutrophil ($\times 10^3$/µL)	0.78 ± 0.21	0.55 ± 0.07	0.58 ± 0.21	0.83 ± 0.13	0.60 ± 0.16
Lymphocyte ($\times 10^3$/µL)	2.38 ± 0.37	2.55 ± 0.36	3.58 ± 1.11	4.10 ± 1.61	2.55 ± 0.55

Table 3. Hematological parameters (at three weeks) of *Wistar* rats administered defatted *Moringa oleifera* seed meal and/or lead acetate

	Group				
Parameter	I (Control)	II (Lead acetate only)	III (Lead acetate +*Moringa oleifera*)	IV (Lead acetate followed by *Moringa oleifera*)	V (*Moringa oleifera* only)
Packed cell volume (%)	40.50 ± 1.32	41.25 ± 1.60	41.50 ± 1.19	41.75 ± 2.50	44.00 ± 1.47
Haemoglobin concentation (g/dL)	13.48 ± 0.44	13.73 ± 0.53	13.80 ± 0.41	13.93 ± 0.82	14.63 ± 0.50
Red blood cells ($\times 10^6/\mu L$)	6.60 ± 0.16	6.85 ± 0.25	6.83 ± 0.24	6.90 ± 0.42	7.28 ± 0.29
MCV mean corpuscular volume (*f*L)	61.36 ± 1.28	60.21 ± 0.22	60.85 ± 0.56	60.52 ± 0.28	60.53 ± 0.54
Mean corpuscular haemoglobin (pg)	20.42 ± 0.42	20.03 ± 0.07	20.23 ± 0.19	20.11 ± 0.08	20.12 ± 0.17
Mean corpuscular haemoglobin concentration (%)	33.27 ± 0.02	33.27 ± 0.02	33.25 ± 0.03	33.24 ± 0.04	33.24 ± 0.04
Total white blood cells ($\times 10^3/\mu L$)	8.23 ± 1.16	9.10 ± 2.18	13.80 ± 3.53	7.43 ± 0.74	10.00 ± 1.12
Neutrophil ($\times 10^3/\mu L$)	0.55 ± 0.10	1.00 ± 0.31	1.73 ± 0.64	1.43 ± 0.22	1.85 ± 0.87
Lymphocyte ($\times 10^3/\mu L$)	7.68 ± 1.09	8.03 ± 1.90	12.03 ± 3.95	5.98 ± 0.59	8.10 ± 0.91

Table 4. Hematological parameters (at four weeks) of *Wistar* rats administered defatted *Moringa oleifera* seed meal and/or lead acetate

	Group				
Parameter	I (Control)	II (Lead acetate only)	III (Lead acetate +*Moringa oleifera*)	IV (Lead acetate followed by *Moringa oleifera*)	V (*Moringa oleifera* only)
Packed cell volume (%)	40.50 ± 1.32	41.50 ± 1.26	41.50 ± 0.96	41.50 ± 2.36	44.25 ± 1.84
Haemoglobin concentration (g/dL)	13.48 ± 0.44	13.80 ± 0.42	13.80 ± 0.31	13.80 ± 0.80	14.70 ± 0.62
Red blood cells ($\times 10^6/\mu L$)	6.80 ± 0.29	6.90 ± 0.22	6.98 ± 0.18	6.78 ± 0.42	7.38 ± 0.30
MCV mean corpuscular volume (*f*L)	59.65 ± 0.96	60.15 ± 0.37	59.51 ± 0.39	61.89 ± 5.10	60.08 ± 1.87
Mean corpuscular hemoglobin (pg)	19.84 ± 0.31	20.00 ± 0.12	19.79 ± 0.13	20.58 ± 1.71	19.96 ± 0.61
Mean corpuscular hemoglobin concentration (%)	33.27 ± 0.02	33.25 ± 0.05	33.25 ± 0.03	33.25 ± 0.05	33.22 ± 0.02
Total white blood cells ($\times 10^3/\mu L$)	6.85 ± 1.14	7.55 ± 0.68	10.90 ± 1.84	7.53 ± 0.52	8.28 ± 0.75
Neutrophil ($\times 10^3/\mu L$)	1.50 ± 0.31	1.33 ± 0.15	2.63 ± 0.64	1.58 ± 0.14	1.88 ± 0.26
Lymphocyte ($\times 10^3/\mu L$)	5.33 ± 1.09	6.13 ± 0.55	8.48 ± 1.45	5.95 ± 0.38	6.33 ± 0.56

Discussion

The results of the 28-day administration study reveal intriguing patterns in hematological parameters across experimental groups. At 7 days, non-significant increases in PCV, Hb, and RBC were observed in groups II, III, IV, and V compared to the negative control (Group I), with the highest values in Group III. This early response may indicate a compensatory mechanism against potential adverse effects (Doig & Zhang, 2017). The lack of significance could be attributed to the relatively short exposure period, as hematological changes might not fully manifest (Doig & Zhang, 2017).

Regarding white blood cell indices, the non-significant decrease in TWBC and lymphocyte count in Group III at 7 days may suggest an initial immunomodulatory effect of the DMOSM (Nfambi et al., 2015; Mohamed et al., 2023). However, the significantly higher neutrophil count in all treatment groups, particularly Group IV, indicates a potential early inflammatory response (Rosales, 2018), possibly triggered by lead acetate exposure. Previous studies have associated lead toxicity with oxidative stress and inflammation (Alhusaini et al., 2019; Abdelhamid et al., 2020; Ilesanmi et al., 2022), and these findings align with those observations.

At 14 days, the sustained non-significant elevation in PCV, Hb, and RBC in all treated groups suggests a persistent influence of lead acetate and DMOSM on red blood cell parameters. The lack of significant differences in MCV, MCH, and MCHC indicates that RBCs' size and hemoglobin content remained relatively stable. The non-significant WBC index increase, especially in Group IV, could imply a prolonged immune response (Nicholson, 2016; Barroso et al., 2021).

At 21 days, the non-significant elevation in PCV, Hb, and RBC persisted across all treated groups, with Group V showing the highest values. This prolonged effect suggests that DMOSM may contribute to sustaining the positive hematological changes initiated by lead acetate exposure. The non-significant WBC index increase in Group III indicates a persistent immunomodulatory effect (Nfambi et al., 2015; Mohamed et al., 2023).

By the end of the 28 days, PCV, Hb, and RBC showed no significant differences across groups but were non-significantly lower in the negative control (Group I). This could indicate a potential protective effect of the administered substances on RBC parameters. The sustained non-significant increase in WBC indices in Group III suggests a continued immunomodulatory effect (Nfambi et al., 2015; Mohamed et al., 2023), aligning with previous observations.

Possible mechanisms behind these results involve the known effects of lead toxicity on hemoglobin synthesis, leading to anemia (Hsieh et al., 2017). *Moringa oleifera* seed meal, rich in antioxidants and nutrients, might counteract these effects, explaining the observed trends in the RBC parameters. In addition, the anti-inflammatory properties of *M. oleifera* could contribute to the observed immune modulation, as suggested by the fluctuations in the WBC indices. Thus, the ability of *M. oleifera* to scavenge free radicals and modulate inflammatory responses could be responsible for the observed patterns in this study.

Conclusion

The present study, therefore, revealed consistent non-significant increases in RBC parameters, particularly PCV, Hb, and RBC, across treated groups compared to the negative control. This sustained elevation suggests a potential compensatory mechanism against lead-induced anemia, possibly influenced by the antioxidant and anti-inflammatory properties of *M. oleifera*. White blood cell indices exhibited non-significant fluctuations, with the combination group (Group III) showing a persistent immunomodulatory effect. These findings imply that DMOSM may play a role in mitigating the adverse hematological effects associated with lead acetate exposure. However, further research is essential to elucidate the underlying mechanisms, validate the observed trends in larger populations, and assess the long-term implications of these effects on overall health.

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