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RESEARCH ARTICLE

Protective Effect of Moringa oleifera Methanolic Leaf Extract on Hematological Parameters, Cortisol, and Malondialdehyde (MDA) Levels in Rats Exposed to Cold Stress

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ABSTRACT

Background: Cold stress is a potent environmental stressor that disrupts hematological homeostasis, activates the hypothalamic–pituitary–adrenal (HPA) axis, and induces oxidative stress. Moringa oleifera is a medicinal plant rich in bioactive compounds with documented antioxidant and adaptogenic properties.

Objective: This study aimed to evaluate the protective effect of methanolic Moringa oleifera leaf extract on hematological parameters, serum cortisol, and malondialdehyde (MDA) levels in rats exposed to cold stress.

Methods: Twenty-four adults male Wistar rats were randomly divided into four groups (n = 6): control, cold stress, M. oleifera extract, and extract plus cold stress. Cold stress was induced by exposure to 4 ± 1 °C for 2 h/day for 15 days. Rats received M. oleifera methanolic leaf extract orally (200 mg/kg body weight). Hematological parameters were assessed using an automated analyzer, while serum cortisol and MDA levels were measured by ELISA. Phytochemical and antioxidant properties of the extract were evaluated using DPPH assay and HPLC analysis.

Results: Cold stress caused significant reductions in red blood cell indices and platelet counts, accompanied by marked elevations in serum cortisol and MDA levels ($p \leqslant 0.05$). Pretreatment with M. oleifera extract significantly ameliorated these alterations, restoring hematological parameters and reducing cortisol and MDA levels toward control values. The extract exhibited strong antioxidant activity and contained notable levels of chlorogenic acid, quercetin, and β-carotene.

Conclusion: Moringa oleifera methanolic leaf extract effectively protects against cold stress–induced hematological, endocrine, and oxidative disturbances in rats, supporting its potential role as a natural antioxidant and adaptogenic agent.

Keywords: Cold stress, Moringa oleifera, HPLC, Cortisol, MDA.

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1. Introduction

Moringa oleifera, commonly known as the drumstick tree, is a highly valued medicinal plant recognized for its rich phytochemical profile and diverse therapeutic properties. It contains abundant bioactive compounds such as flavonoids, phenolic acids, vitamins C and E, and carotenoids, which contribute to its potent antioxidant, anti-inflammatory, and hematoprotective activities [1]. The leaves of *M. oleifera* are particularly rich in natural antioxidants that scavenge free radicals, reduce lipid peroxidation, and enhance the body's defense mechanisms against oxidative damage. In addition to its antioxidant potential, *M. oleifera* has been reported to possess immunomodulatory, antihyperglycemic, hepatoprotective, and adaptogenic properties, making it an effective natural agent for combating physiological and environmental stressors [2,3].

Natural adaptogens like *M. oleifera* play an important role in maintaining internal homeostasis and improving the body's resistance to physical, chemical, or biological stress [4]. Regular intake of *M. oleifera* extract has been shown to enhance antioxidant enzyme activity, regulate hormonal responses, and stabilize hematological parameters under various stress conditions [5].

Stress, in general, is defined as an organism's total physiological and biochemical response to environmental pressures that threaten homeostasis [6]. When exposed to stress, the body activates an adaptive mechanism involving the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the secretion of cortisol—the primary stress hormone [7]. The key biological mediator of stress is cortisol, which is a glucocorticoid hormone synthesized and released by the adrenal glands in response to stressors. The cortisol hormone plays a central role in the body's "fight or flight" response by mobilizing energy, regulating metabolism, and modulatory inflammation [8].

Cold stress represents one of the most potent environmental stressors that induce significant physiological and metabolic alterations in mammals. Prolonged exposure to low temperatures stimulates the HPA axis, elevating cortisol secretion and generating excessive reactive oxygen species (ROS), which cause oxidative stress and cellular injury [9]. Among the biochemical indicators of oxidative damage, malondialdehyde (MDA) is considered a reliable marker of lipid peroxidation and membrane damage [10]. Elevated MDA levels reflect enhanced ROS generation and impaired antioxidant defense mechanisms during cold exposure [11]. Consequently, simultaneous evaluation of cortisol and MDA levels provides valuable insight into the stress-induced endocrine and oxidative responses in animals.

Given its well-documented antioxidative and adaptogenic properties, *Moringa oleifera* may provide significant protection against cold stress-induced hematological and biochemical disturbances [12]. However, limited research has addressed its efficacy under such conditions, particularly regarding alterations in cortisol and malondialdehyde (MDA) levels. Therefore, this study aimed to evaluate the protective effects of methanolic *M. oleifera* leaf extract on hematological parameters, serum cortisol, and MDA levels in male rats exposed to cold stress for 15 days, to elucidate its potential antioxidant and adaptogenic mechanisms in maintaining hematological and endocrine stability.

2. Materials and methods

2.1. Plant collection, identification and drying

Fresh *Moringa oleifera* leaves were collected locally from a private farm house in Hillah, Babylon, Iraq, and identified by Dr. Nidaa Adnan (Plant herbarium / department of biology / college of science / university of Babylon) washed, shade-dried, and finely powdered and kept in a closed and sterile container.

2.2. Preparation of Moringa oleifera Methanolic Leaf Extract

The powdered leaves were extracted according to [13] by mixing 1 gm of raw *Moringa* leaf powder with 10 ml of the methanol-water solvent. The mixture was shaken at high speed for 1 hour to ensure proper mixing, then incubated for two hours at 40°C in a water bath. The suspension was filtered by guise and the filtrated liquid was concentrated to dryness in oven at 45 C°. The dried and concentrated material was ground into a fine powder and sterilized under UV light for 20 minutes.

2.3. DPPH Radical Scavenging Activity Test

The antioxidant activity of *Moringa oleifera* methanolic leaf extract was evaluated using the DPPH radical scavenging assay. Different extract concentrations (3.125–200 µg/mL) were incubated for 30 min, and absorbance was measured at 517 nm. All measurements were performed in triplicate. The percentage scavenging activity was calculated as:

$$Q = 100 \times (A0 - AC) / A0$$

where A0 is the control absorbance and AC is the sample absorbance. The IC50 value was determined from the concentration–response curve [14,15].

2.4. HPLC Analysis

Reversed-phase HPLC was performed using a C18 column (25 cm × 4.6 mm, 5 µm). Phenolic compounds (chlorogenic acid and quercetin) were separated using a gradient system of 0.25% orthophosphoric acid (A) and acetonitrile (B), while β-carotene was analyzed using a methanol–acetic acid–water gradient system. The flow rate was 1.0 mL/min, injection volume 100 µL, and column temperature 25 °C. Detection wavelengths were 360 nm for phenolics and 280 nm for β-carotene, following previously validated methods.

2.5. Experimental animals

Twenty-four adults male Wistar albino rats weighing (200–300 gm), were obtained from the animal house in Najaf Governorate and housed in the animal house of the Biology Department, College of Science, University of Babylon. The animals were housed under standard laboratory conditions (temperature 22 ± 2 °C; 12 h light/dark cycle) with free access to a standard pellet diet and water and they were given time to adapt for approximately two weeks. During this period, the rats were given water *ad libitum* and fed a standard pellet diet.

2.6. Experimental Design

The experiment was conducted for 15 days to evaluate the protective effect of *Moringa oleifera* methanolic leaf extract against cold stress–induced physiological changes. The rats were randomly divided into four groups (n = 6 each) as follows:

Group	Treatment Description
Control	Rats maintained at room temperature and given distilled water orally.
Cold Stress (Stress)	Rats exposed to cold stress (4 ± 1 °C) for 2 h/day for 15 days; received distilled water.
Moringa Extract (Extract)	Rats maintained at room temperature and administered *Moringa oleifera* methanolic leaf extract (200 mg/kg b.wt, orally) for 15 days.
Extract + Cold Stress (Protective)	Rats pretreated with *M. oleifera* extract (200 mg/kg b.wt, orally) for 15 days, then exposed to cold stress (4 ± 1 °C, 2 h/day) for another 15 days

Body weight and clinical signs were monitored throughout the experimental period.

2.7. Cold stress models

Cold stress was the stress paradigm used in this study, as previously stated by [16]. Cold stress was induced by placing the rats individually in an isolated containers with ice cubes to maintain a temperature of 4°C for 2 hours daily between 8:00 and 10:00 a.m. in order to prevent interference with the with the corticosterone circadian rhythm for 15 consecutive days. Each rat was returned to room temperature immediately after exposure and observed for signs of distress. Control and extract-only groups were kept at ambient room temperature (22 ± 2 °C).

2.8. Hematological Analysis

Hematological parameters were determined using the Mythic 18 VET, a fully automated benchtop analyzer for hematology that uses impedance technology.

2.9. Determination of Serum Cortisol and MDA

Serum cortisol and MDA concentrations were measured using a rat-specific ELISA kit (Elabscience®, China) following the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader. Cortisol and MDA levels were expressed in ng/mL.

2.10. Statistical analysis

The collected data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 23.0 (IBM Corp., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered statistically significant at ($P \leq 0.05$).

3. Results

3.1. Phytochemical Analysis Study

3.1.1. DPPH Scavenging Activity Assay

The antioxidant activity of Moringa oleifera methanol - watery extract was measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity test and the results in Table (1) showed dose dependent of antioxidant activity of the extract reached to (90%) in the concentration 200) µg/ml with IC50 (47.89) µg/mL.

Table 1. The percentage of DPPH scavenging activity for different concentrations of *M. oleifera* Methanolic extract.

No.	Concentration µg/ml	Absorption		Mean	Scavenging Activity %
		R1	R2		
1	0	0.85	0.85	0.85	0
2	3.125	0.68	0.67	0.67	21
3	6.25	0.55	0.53	0.54	36
4	12.5	0.47	0.49	0.48	43
5	25	0.35	0.37	0.36	57
6	50	0.21	0.24	0.22	74
7	100	0.14	0.12	0.13	84
8	200	0.09	0.08	0.08	90

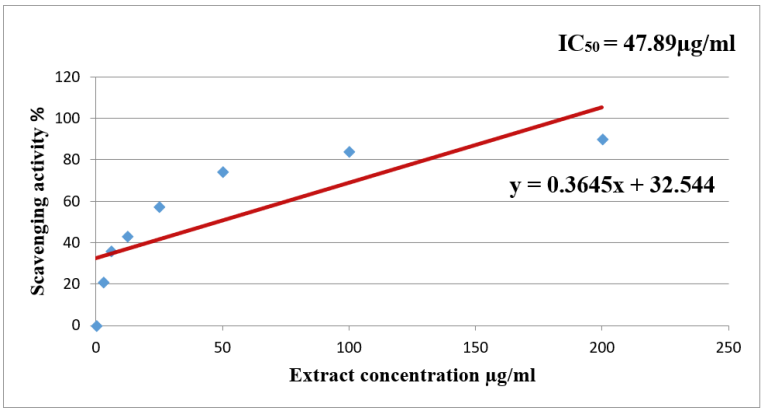
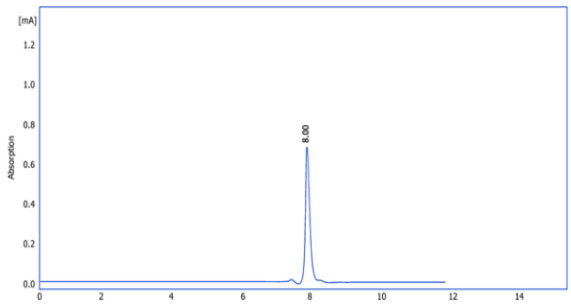


Figure 1. Response curve of different concentrations of *M. oleifera* methanolic extract on DPPH free radical scavenging activity.

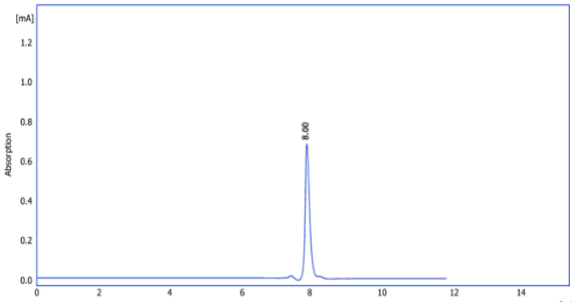
3.2. HPLC Analysis

3.2.1. Quantitative and Qualitative Identification of Chlorogenic acid in M. oleifera by HIPLC

The presence and concentration of Chlorogenic acid was identified by conducting HPLC analysis in a suitable condition for the stationary phase and a graded polarity system for the mobile phase, as shown in Figure (2), where the retention time for M. oleifera was 8.00 and the area was 9832.14 compared to the retention time for Chlorogenic and the area which was 8.00 minutes, 1457.90 respectively. This result showed the presence of Chlorogenic substance at a concentration of 98.2 ppm.



Chlorogenic acid sample



***M. oleifera* extract**

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	8.00	1457.90	745.14	100.00	100.00	0.25	
	Total	1457.90	745.14	100.00	100.00		

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	3.05	9854.08	800.8	25.00	25.00	0.08	
2	5.14	5241.11	408.78	13.00	13.00	0.04	
3	6.00	15426.98	998.41	27.00	27.00	0.10	
4	8.00	9832.14	806.08	25.00	25.00	0.08	
5	9.80	3202.58	310.25	10.00	10.00	0.02	
	Total	43556.29	3323.16	100.00	100.00		

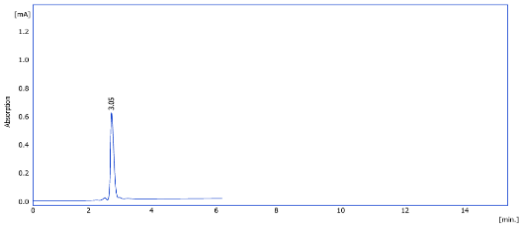
Chlorogenic con = 98.2 ppm

Con. = Concentration

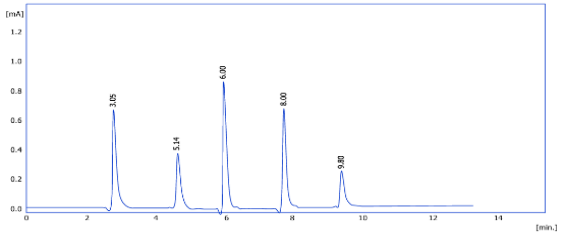
Figure 2. HPLC analysis for chlorogenic acid in methanolic extract of *Moringa oleifera.*

3.2.2. Quantitative and Qualitative Identification of Quercetin in M. oleifera by HIPLC

The presence and concentration of Quercetin was identified by performing HPLC analysis in a proper condition of stationary phase and a step gradient polarity system of mobile phase as shown in figure (3), where the retention time of M. oleifera extract was 3.05 min and area was 9854.08 in compared with Quercetin standard retention time and area which was respectively 3.05 min and 1245.90. This result revealed the presence of Quercetin in concentration 77.4 ppm.



Quercetin sample



***M. oleifera* extract**

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	3.05	1245.90	605.90	100.00	100.00	0.25	
	Total	1245.90	605.90	100.00	100.00		

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	3.05	9854.08	800.8	25.00	25.00	0.08	
2	5.14	5241.11	408.78	13.00	13.00	0.04	
3	6.00	15426.98	998.41	27.00	27.00	0.10	
4	8.00	9832.14	806.08	25.00	25.00	0.08	
5	9.80	3202.58	310.25	10.00	10.00	0.02	
	Total	43556.29	3323.16	100.00	100.00		

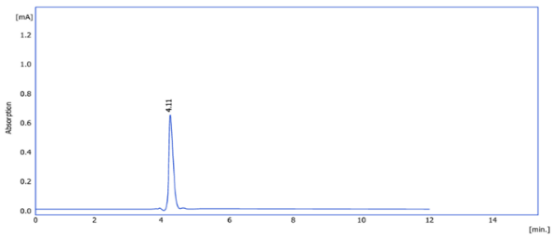
Quercetin con = 77.4 ppm

Figure 3. HPLC analysis for quercetin in methanolic extract of *Moringa oleifera*.

3.2.3. Quantitative and Qualitative Identification of β - Carotene in M. oleifera by HIPLC

The presence and concentration of β- Carotene were identified by conducting HPLC analysis in a suitable condition for the stationary phase and a graded polarity system for the mobile phase, as shown in Figure (4),

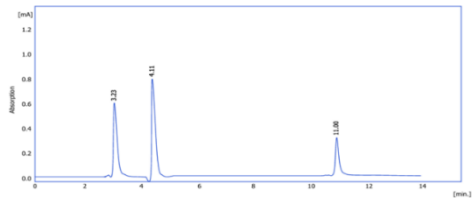
where the retention time for *M. oleifera* was 4.11 and the area was 6521.44 compared to the retention time for β- Carotene and the area which was 4.11 minutes, 2032.66 respectively. This result showed the presence of β-Carotene in concentration of 87.9 ppm.



β- Carotene sample

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	4.11	2032.66	633.15	100.00	100.00	0.25	
	Total	2032.66	633.15	100.00	100.00		

β – Carotene con = 87.9 ppm



***M. oleifera* extract**

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [%]	Compound Name
1	3.23	2541.65	600.08	40.00	40.00	0.15	
2	4.11	6521.44	791.24	50.00	50.00	0.20	
3	11.00	541.98	320.65	10.00	10.00	0.10	
	Total	9605.14	1711.19	100.00	100.00		

Figure 4. HPLC analysis for β – Carotene in methanolic extract of *Moringa oleifera*.

3.3. The protective effect of M. oleifera extract on some hematological parameters in rats exposed to cold stress for 15 days.

Table (2) presents the effects of *Moringa oleifera* methanolic leaf extract on hematological parameters in rats exposed to cold stress for 15 days. Cold stress exposure for 15 days caused significant alterations in hematological parameters (Table 1). The RBC count, hemoglobin concentration (HGB), and hematocrit value (HCT) were markedly decreased ($p < 0.05$) in the Cold Stress group ($3.58 \pm 0.8 \times 10^6/\mu L$, 8.38 ± 1.0 g/dL, and $30.07 \pm 1.5\%$, respectively) compared to the Control group ($7.50 \pm 0.7 \times 10^6/\mu L$, 13.12 ± 1.7 g/dL, and $47.47 \pm 3.4\%$). Pretreatment with M. oleifera methanolic leaf extract prior to cold exposure (Extract + Cold Stress) markedly improved these parameters, recording values of $5.10 \pm 0.6 \times 10^6/\mu L$, 8.33 ± 1.9 g/dL, and $30.93 \pm 4.3\%$, respectively. Administration of the extract alone (Extract group) significantly increased RBC count, HGB, and HCT ($9.92 \pm 1.2 \times 10^6/\mu L$, 14.57 ± 2.5 g/dL, and $54.38 \pm 7.6\%$) compared to the control.

Indices of red cell morphology also showed similar trends. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly reduced ($p < 0.05$) in the Cold Stress group (46.18 ± 6.4 fL, 12.83 ± 1.8 pg, and 20.62 ± 1.4 g/dL, respectively) relative to the control (56.43 ± 5.1 fL, 17.37 ± 1.2 pg, and 32.50 ± 2.2 g/dL). Pretreatment with M. oleifera markedly ameliorated these reductions, as shown by increased values in the Extract + Cold Stress group (56.88 ± 2.2 fL, 16.05 ± 2.7 pg, and 28.23 ± 2.9 g/dL). The Extract group alone exhibited the highest readings (63.95 ± 4.6 fL, 22.65 ± 3.5 pg, and 36.72 ± 2.1 g/dL), reflecting the hematopoietic potential of M. oleifera.

Furthermore, red cell distribution width (RDW) significantly increased ($p < 0.05$) in the Cold Stress group ($21.72 \pm 3.1\%$) compared to the Control ($16.58 \pm 1.1\%$), indicating red cell size variation due to stress-induced erythrocyte damage. Pretreatment with M. oleifera reduced RDW values ($14.78 \pm 2.2\%$) toward normal. Platelet count (PLT) was also markedly decreased in the Cold Stress group ($356.00 \pm 17.4 \times 10^3/\mu L$) compared to the Control ($609.67 \pm 15.5 \times 10^3/\mu L$), while the Extract + Cold Stress group showed a partial restoration ($222.33 \pm 34.6 \times 10^3/\mu L$). The Extract group alone displayed the highest platelet level ($799.83 \pm 10.7 \times 10^3/\mu L$), further supporting the stimulatory effect of M. oleifera on hematopoiesis.

Table 2. The protective effect of *M. oleifera* extract on some hematological parameters (Mean ± SD) in rats exposed to cold stress for 15 days.

Parameters \ Groups	Control	Cold Stress	Extract	Extract + Cold Stress
		Mean±S.D		
RBC 10^6/µl	7.50±0.7 c	3.58±0.8 a	9.92±1.2 d	5.1±0.6 b
HGB g/dL	13.12±1.7 b	8.38±1.0 a	14.57±2.5 b	8.33±1.9 a
HCT %	47.47±3.4 bc	30.07±1.5 a	54.38±7.6 c	30.93±4.3 a
MCV fL	56.43±5.1 b	46.18±6.4 a	63.95±4.6 c	56.88±2.2 b
MCH Pg	17.37±1.2 b	12.83±1.8 a	22.65±3.5 c	16.05±2.7 b
MCHC g/dL	32.50±2.2 b	20.62±1.4 a	36.72±2.1 c	28.23±2.9 b
RDW %	16.58±1.1 b	21.72±3.1 c	11.48±1.4 a	14.78±2.2 a
PLT 10^3/ µl	609.67±15.5 c	356.00±17.4 b	799.83±10.7 d	222.33±34.6 a

Different letters mean significant difference at ($p \leq 0.05$).

RBC= Red blood cells, HGB= Hemoglobin, HCT=Haematocrit, MCV= Mean corpuscle volume, MCH= Mean corpuscle hemoglobin, MCHC= Mean corpuscle hemoglobin concentration, RDW= Red blood distribution width and PLT= Platelets

3.4. The protective effect of M. oleifera extract on serum cortisol level (Mean ± SD) in rats exposed to cold stress for 15 days.

Cold stress exposure for 15 days resulted in significant elevations in serum cortisol levels compared to the control group (Figure 5). The Cold Stress group showed a marked increase ($p < 0.05$) in cortisol level (37.26 ± 6.9 ng/mL) relative to the Control (15.10 ± 1.9 ng/mL), indicating activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to stress. Pretreatment with Moringa oleifera methanolic leaf extract prior to cold exposure (Extract + Stress group) significantly reduced cortisol concentration (18.09 ± 2.8 ng/mL), approaching the normal range. Administration of the extract alone (Extract group) produced the lowest cortisol value (8.40 ± 0.4 ng/mL), suggesting a potential stress-attenuating and adaptogenic effect of the extract.

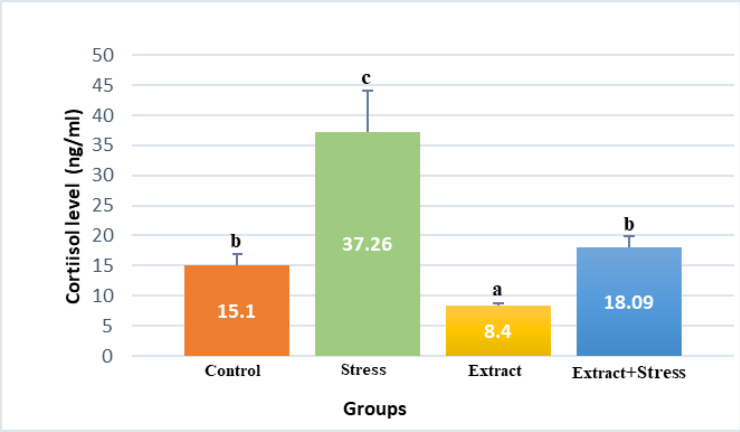


Figure 5. The protective effect of *M. oleifera* extract on serum cortisol levels in rats exposed to cold stress for 15 days.

3.5. The protective effect of M. oleifera extract on serum cortisol level (Mean ± SD) in rats exposed to cold stress for 15 days.

Figure (6) demonstrates the MDA levels, which reflect lipid peroxidation and oxidative stress, were significantly elevated in the Cold Stress group (1754.27 ± 15.6 nmol/L) compared to the Control (1143.44 ± 53.3 nmol/L). Pretreatment with M. oleifera markedly reduced MDA concentration (1085.2 ± 24.7 nmol/L),

indicating effective protection against oxidative damage. Rats receiving M. oleifera extract alone (910.03 ± 114.6 nmol/L) maintained the lowest MDA value, comparable to or below control levels.

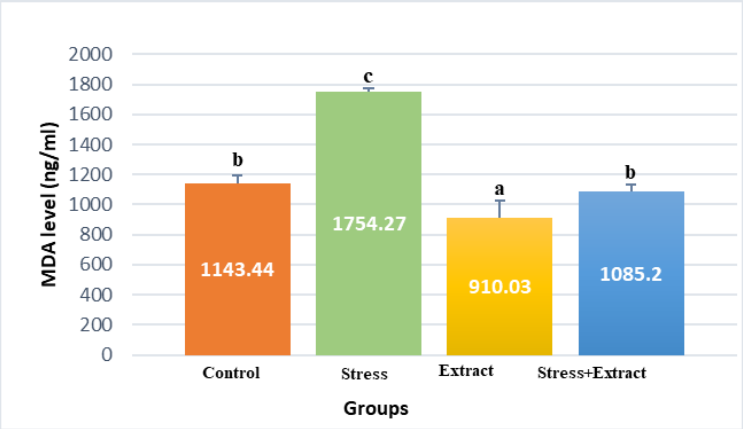


Figure 6. The protective effect of *M. oleifera* extract on serum MDA levels in rats exposed to cold stress for 15 days.

4. Discussion

Cold stress triggers oxidative imbalance, hormonal disruption, and hematological alterations. In this study, cold exposure significantly increased serum cortisol and MDA levels while impairing hematological parameters, confirming oxidative and metabolic stress via HPA axis activation and excessive ROS production. Pretreatment with *Moringa oleifera* methanolic leaf extract effectively ameliorated these changes, reflecting strong antioxidative and adaptogenic activity. These protective effects are attributed to bioactive constituents such as chlorogenic acid, quercetin, and β-carotene, which enhance free radical scavenging and endocrine stability. Overall, *M. oleifera* demonstrates potent hematoprotective and antioxidant potential against cold stress–induced disturbances [17]. The DPPH assay is a simple and reliable method for assessing antioxidant activity, as the stable DPPH radical shows a decrease in absorbance at 517 nm when reduced by hydrogen or electron-donating compounds [18]. In this study, *Moringa oleifera* extract demonstrated notable DPPH radical-scavenging activity, indicating its ability to donate hydrogen atoms and neutralize free radicals. This antioxidant effect reflects the presence of bioactive constituents capable of protecting cells from oxidative damage [19]. The phytochemical profile of *M. oleifera*, particularly its phenolic and flavonoid content, likely contributes to the observed radical-scavenging capacity [20]. The detection of chlorogenic acid in *M. oleifera* leaf extract further confirms the plant's rich phenolic profile. In this study, chlorogenic acid was identified at a retention time of 8.00 minutes—matching the standard—and showed a markedly higher peak area (9832.14) than the standard (1457.90), corresponding to a concentration of 98.2 ppm. This aligns with previous reports describing chlorogenic acid as a major phenolic constituent of *M. oleifera* and an important contributor to its antioxidant potential [21]. Chlorogenic acid, a well-known polyphenol, exhibits strong antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective activities, partly through modulation of the NF-κB signaling pathway [22]. The presence of chlorogenic acid alongside other phenolics such as gallic and ellagic acids supports the notion that the extract's biological activity results from the synergistic effects of multiple compounds, which may collectively underpin the observed antioxidant and cytoprotective properties of *M. oleifera* [23]. The identification of quercetin in the methanolic leaf extract of *M. oleifera* reinforces the plant's status as a rich source of bioactive flavonoids. In this study, quercetin was detected at a retention time of 3.05 minutes, matching the standard, and exhibited a high peak area (9854.08) corresponding to a concentration of 77.4 ppm. This agrees with earlier studies that consistently report quercetin as one of the major phenolic constituents of *M. oleifera* [24]. Quercetin is a potent flavonoid with strong free radical–scavenging capacity, effectively preventing oxidative damage to lipids, DNA, and cellular components. Its broad pharmacological activities—including anti-inflammatory, antidiabetic, cardioprotective, and anticancer effects—further

highlight its therapeutic relevance [25]. Previous reports also show that dried *M. oleifera* leaves contain substantial amounts of quercetin derivatives, such as quercetin-3-O-β-D-glucoside, supporting the high antioxidant potential of the plant [26]. HPLC analysis confirmed the presence of β-carotene in *Moringa oleifera* leaves, with a retention time of 4.11 minutes matching the standard and a quantified concentration of 87.9 ppm. The corresponding peak area values validate this identification and highlight *M. oleifera* as a rich source of carotenoids, consistent with earlier reports describing its high β-carotene content and provitamin A value [27]. β-carotene functions both as a potent antioxidant—scavenging reactive oxygen species—and as a precursor of vitamin A, which is essential for vision, immune function, and epithelial health [28]. The substantial β-carotene level detected in *M. oleifera* underscores its nutritional significance and supports its use as a dietary supplement, particularly in populations vulnerable to vitamin A deficiency [29].

This study evaluated the impact of *Moringa oleifera* methanolic leaf extract on hematological changes in rats exposed to cold stress for 15 days. As reported in previous research, cold exposure significantly disturbed hematological homeostasis, causing reductions in RBC, HGB, HCT, MCV, MCH, MCHC, and PLT, along with increased RDW [30]. These alterations indicate anemia and impaired erythropoiesis, likely resulting from oxidative damage and bone marrow suppression [31,32]. *M. oleifera* administration markedly improved these hematological disturbances. The extract restored RBC indices, normalized RDW, and increased platelet counts in cold-stressed rats. Treatment with the extract alone produced hematological values higher than the control group, reflecting its strong hematopoietic potential. These effects may be attributed to the plant's rich antioxidant and nutrient content—including flavonoids, phenolics, vitamins, and iron—which support erythropoiesis and protect against oxidative injury [33,34]. The protective action of *M. oleifera* is likely mediated by its antioxidant and adaptogenic properties, which reduce ROS formation, enhance endogenous antioxidant enzymes, and preserve bone marrow function [35,36]. The improvement in platelet levels further suggests enhanced thrombopoiesis.

Cold stress exposure for 15 days resulted in a significant elevation of serum cortisol levels, confirming activation of the hypothalamic–pituitary–adrenal (HPA) axis as a central stress-response mechanism [16]. The marked increase in cortisol observed in the Cold Stress group compared with controls reflects endocrine adaptation to sustained metabolic and thermogenic demands imposed by cold exposure, which stimulate hypothalamic corticotropin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH) secretion [37,38]. Similar cortisol elevations have been reported in sub chronic cold stress models and are associated with glucocorticoid-mediated physiological disturbances during prolonged stress exposure [39].

In addition to its endocrine effects, sustained HPA axis activation contributes to metabolic acceleration and increased mitochondrial activity, processes that are closely linked to enhanced production of reactive oxygen species (ROS). Consistent with this interaction between endocrine and oxidative stress pathways, cold stress exposure was accompanied by a significant elevation in serum malondialdehyde (MDA) levels, indicating increased lipid peroxidation and disruption of redox homeostasis. MDA is a well-established marker of oxidative membrane damage generated during ROS-mediated degradation of polyunsaturated fatty acids [40]. The marked increase observed in the Cold Stress group reflects excessive ROS generation during cold exposure.

Cold stress is known to elevate metabolic demand and sympathetic nervous system activity, thereby increasing mitochondrial oxygen consumption and ROS production [41]. When endogenous antioxidant defenses are insufficient to counteract this oxidative burden, lipid peroxidation intensifies, leading to increased circulating MDA levels, as previously reported in cold stress models [42].

Pretreatment with *Moringa oleifera* methanolic leaf extract markedly attenuated both cortisol elevation and the cold stress–induced rise in MDA levels, with values approaching those of the control group. Suppression of cortisol suggests modulation of HPA axis hyperactivation, while the reduction in MDA indicates effective protection against oxidative membrane damage [43]. Administration of the extract alone

resulted in the lowest cortisol and MDA levels, suggesting that *M. oleifera* supports basal endocrine balance and possesses strong intrinsic antioxidant activity under normal physiological conditions [44]. The protective effects of *M. oleifera* are attributed to its rich phytochemical composition, including polyphenols, flavonoids, vitamins, and carotenoids, which synergistically scavenge free radicals, inhibit lipid peroxidation, and reinforce endogenous antioxidant defenses [36]. Collectively, these findings demonstrate that *Moringa oleifera* extract effectively mitigates cold stress–induced endocrine and oxidative disturbances after 15 days of exposure, supporting its potential as a natural adaptogenic and antioxidant agent. This also positions cold stress as an environmental stress model in living systems, where the protective effects of *Moringa oleifera* highlight the role of natural antioxidants in stress mitigation. These findings further align with green chemistry and environmental chemical engineering principles through the use of plant-based, eco-friendly bioactive compounds.

5. Conclusion

The present study demonstrates that chronic cold stress induced significant hematological, endocrine, and oxidative disturbances in rats, as evidenced by altered blood indices and elevated cortisol and MDA levels, indicating activation of the HPA axis and increased lipid peroxidation.

Moringa oleifera methanolic leaf extract effectively alleviated these changes, restoring hematological parameters and reducing cortisol and MDA levels, demonstrating strong antioxidant and adaptogenic properties likely linked to its phytochemical content.

Overall, *M. oleifera* shows potential as a natural protective agent against cold stress–induced physiological dysfunction. In addition, its extraction process could be improved using green chemistry approaches, including solvent reduction, recycling, and energy-efficient processing, to enhance sustainability and environmental safety. Its phytochemical constituents may also offer potential applications in green nanoparticle synthesis as reducing and capping agents, representing a future research direction.

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Ethics Approval and Consent to Participate

All experimental protocols involving animals were conducted according to the ethical standards for animal research and were approved by the Animal Ethics Committee of the College of Science, University of Babylon, Department of Biology (Approval No. Z240104, January 30, 2024).

Competing Interests

The authors declare that there is no conflict of interest.

Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented to it being published.

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