

Protective role of *Moringa oleifera* seed nanoparticles against lipid metabolism disturbances, oxidative stress, and immune dysregulation in diet-induced hyperlipidemic Wistar rats

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ABSTRACT

This study investigated the protective effects of *Moringa oleifera* seed nanoparticles (MO-SeNPs) against high-fat diet-induced hyperlipidemia in Wistar rats. Rats were allocated into normal control, high-fat diet (HC) control, and HC groups receiving MO-SeNPs (25, 100, or 200 mg/kg) for 60 days. The HC group showed significant dyslipidemia, oxidative stress (elevated MDA, decreased SOD/CAT), and immune dysregulation (increased TNF- α /IL-6, decreased IL-10). MO-SeNP supplementation, particularly at 200 mg/kg, dose-dependently reversed these alterations, restoring lipid profiles, oxidative balance, and cytokine levels to near-normal. Results demonstrate that MO-SeNPs effectively ameliorate hyperlipidemia-induced metabolic disturbances, highlighting their potential as a natural therapeutic agent.

Keywords: *Moringa oleifera*, lipid metabolism, oxidative stress, inflammatory responses, hyperlipidemic Wistar rats.

INTRODUCTION

Hyperlipidemia is characterized by elevated levels of low-density lipoproteins, triglycerides, and cholesterol, and is a major metabolic disorder often accompanied by reduced levels of high-density lipoproteins (Yao et al., 2020). It represents a primary risk factor for myocardial infarction, stroke, and atherosclerosis, which cause substantial global morbidity and mortality (Herrington et al., 2016). In addition to lipid dysregulation, Hyperlipidemia also contributes to metabolic dysfunctions, systemic swelling, and oxidative stress, which worsen organ damage (Samar et al., 2022). The liver plays a vital role in lipid metabolism with the storage, synthesis, and catabolism of lipids (Bechmann et al., 2012). Under hyperlipidemic situations, extreme lipid growth causes non-alcoholic fatty liver disease that can lead to steatohepatitis, cirrhosis, and fibrosis (Pappachan et al., 2017; Al Sulivany and Hoseini, 2025). Lipid excess in the liver promotes excessive production of reactive oxygen species, leading to oxidative stress and disrupting antioxidant defense mechanisms (Banerjee et al., 2020). This oxidative disparity enables LDL oxidation, which is a primary trigger of pro-inflammatory signaling. Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and other cytokines contribute to systemic inflammation and cause tissue injury (García et al., 2022). Similarly, hyperlipidemia impairs renal function through oxidative stress and lipid accumulation, leading to decreased glomerular filtration rate, interstitial fibrosis, and glomerular impairment (Karanovic et al., 2021; Zulfiqar et al., 2025). These uncontrolled progressions include the initiation of signaling pathways, such as those involving inflammatory mediators and nuclear factor kappa B (NF- κ B) (Guo et al., 2024). The interaction between oxidative stress and inflammation thus forms a brutal cycle that impairs organ dysfunction in hyperlipidemia (Matyas et al., 2021). Pharmacological methods for treating hyperlipidemia are effective in reducing cardiovascular risk and lowering lipid levels. However, long-term use can cause adverse effects such as myopathy, impaired glucose metabolism, and hepatotoxicity (Marini et al., 2015). These limitations have prompted growing interest in discovering alternative treatments, particularly those derived from natural sources, which often offer multi-targeted effects with fewer side effects (Singh et al., 2024).

Moringa oleifera, commonly known as the drumstick tree, has attracted significant attention for its medicinal properties and a rich profile of bioactive compounds (Kashyap et al., 2022). Its seeds are an important source of flavonoids, phenolic acids, isothiocyanates, and other phytochemicals, which have been identified for their lipid-lowering effects, antioxidant, and anti-inflammatory properties (Shahbaz et al., 2024). *M. oleifera* seed extracts can ameliorate hyperlipidemia by increasing high-density lipoprotein (HDL) while decreasing the triglycerides, total cholesterol, and LDL (Alkhudhayri et al., 2021). Moreover, these extracts demonstrated nephroprotective and

hepatoprotective properties by modulating inflammatory pathways and oxidative stress (Kukreti et al., 2023). Despite the beneficial properties of *M. oleifera* seeds, their medical applications are constrained by rapid degradation in the gastrointestinal tract, reduced bioavailability, and limited target specificity (Chhikara et al., 2021). Nanotechnology offers auspicious results to these limitations by enhancing the targeted delivery of plant-derived bioactives, solubility, and stability (Karnwal et al., 2024). Nano-formulations can extend systemic circulation and absorption, thus potentiating therapeutic properties (Wahi et al., 2023; Fazal et al., 2025). Phytochemical nanoparticles exhibit better lipid-lowering, antioxidant, and anti-inflammatory properties than their crude extracts (Siddique et al., 2024). Despite rising interest in nanoformulations of plant extracts, few studies have addressed the potential role of *M. oleifera* seed nanoparticles (MO-SeNPs) in the management of hyperlipidemia. Existing studies have mainly focused on conventional extracts rather than their nano-enabled counterparts, leaving a critical gap in knowledge regarding their comparative efficacy and mechanisms of action (Adisa et al., 2019; Owais et al., 2025). Given these gaps, the present study aims to assess the protective effects of MO-SeNPs on oxidative stress, lipid metabolism, liver and kidney function, and immune-inflammatory responses in diet-induced hyperlipidemic Wistar rats.

MATERIALS AND METHODS

Study site and ethical approval

This study was conducted at the Department of Zoology, Government College University Lahore, Pakistan, under controlled environmental conditions. All measures involving animals complied with the rules and were accepted by the Institutional Animal Ethics Committee of the Government College University of Lahore.

Experimental animals and housing

Wistar rats (*Rattus norvegicus*) were obtained from the Department of Zoology, Government College University Lahore animal house. 120 male *R. norvegicus* weighing 150–200 g and aged 8–10 weeks were taken, acclimatized, and maintained for one week before the experiment under standard laboratory conditions such as 22 ± 2 °C, 50–60% relative humidity, 12 h light/dark cycle. Clean drinking water and feed were given throughout the experiment, except for the fasting period before sampling. Polypropylene cages with sterile paddy husk bedding were provided for housing, and bedding was changed twice weekly to maintain hygienic conditions (Samar et al., 2022).

Experimental Design and Diet Formulation

Five experimental groups were designed, each with three replicates, and a total of 120 rats ($n = 8$ per replicate, total 24 rats per group) were randomly assigned to the groups for a 60-day study. The first group received standard laboratory chow with 0.5% carboxymethyl cellulose (CMC) as the vehicle, designated the standard control (NC) group.

The second group was designated a high-fat control (HC) group and received a high-fat diet (HFD) to induce hyperlipidemia, along with 0.5% CMC (Carboxymethyl Cellulose). The third group with HFD supplemented with *M. oleifera* seed nanoparticles was designated the low-dose MOS-NP-L (25 mg/kg body weight/day). The fourth group, HFD supplemented with *Moringa oleifera* seed nanoparticles, was designated the medium-dose MOS-NP-M (100 mg/kg body weight/day). The fifth group, HFD supplemented with *Moringa oleifera* seed nanoparticles, was designated as the high-dose MOS-NP-H (200 mg/kg body weight/day). Freshly prepared MOS-NPs were suspended in 0.5% CMC each day and administered orally by gavage at 10 mL/kg body weight to ensure precise dosing. The HFD was prepared following the composition described by Buettner et al. (2007), containing 20% protein, 35% carbohydrates, and 45% fat. Diets were freshly prepared and stored in airtight containers at 4 °C. The composition of the experimental diet is presented in Table 1.

Table 1. Composition of experimental diets

Diet Group	Fat (%)	Protein (%)	Carbohydrate (%)	Additives
Normal Control (NC)	10	20	70	0.5% CMC
High-Fat Control (HC)	45	20	35	0.5% CMC
MOS-NP-L	45	20	35	MOS-NPs 25 mg/kg BW/day + 0.5% CMC
MOS-NP-M	45	20	35	MOS-NPs 100 mg/kg BW/day + 0.5% CMC
MOS-NP-H	45	20	35	MOS-NPs 200 mg/kg BW/day + 0.5% CMC

Sample Collection

After the end of the sixty-day experimental period, all animals were subjected to an overnight fast (12 h) to minimize the impact of recent feeding

(Al Sulivany and Al Habbib, 2013). After that, the animals were anesthetized by intraperitoneal injection (ketamine, 80 mg/kg BW, and xylazine, 10 mg/kg BW), followed by sacrifice via cardiac puncture. Blood samples were collected, allowed to clot, and centrifuged at 3000 rpm for 15 min at 4 °C (El-Demerdash and Nasr, 2014). After blood collection, the major organs (liver, kidney, spleen, and epididymal fat pads) were immediately removed, washed gently in ice-cold saline to remove excess blood, blotted dry on filter paper, and weighed using an analytical balance. Tissue samples for subsequent biochemical assays (oxidative stress and lipid metabolism markers) were stored at -80 °C (Tiwari et al., 2018).

Lipid Profile

Serum lipid parameters were examined by enzymatic colorimetric kits (BioSystems, Spain). Total cholesterol (TC) was measured by the cholesterol oxidase-peroxidase method, and triglycerides (TG) were assessed by the glycerol-3-phosphate oxidase method, both of which are well-documented, consistent, and sensitive diagnostic procedures (Friedewald et al., 1972; Allain et al., 1974). High-density lipoprotein (HDL) was measured after the selective precipitation of non-HDL lipoproteins, followed by enzymatic determination of the supernatant fraction (Gordon et al., 1977; Al Sulivany et al., 2025). The Friedewald Equation calculated very-low-density lipoprotein (VLDL) and Low-density lipoprotein (LDL):

$$\begin{aligned}\text{LDL (mg/dL)} &= \text{TC} - \text{HDL} - \text{C} - \text{TG}/5 \\ \text{VLDL (mg/dL)} &= \text{TG}/5\end{aligned}$$

TG levels were under 400 mg/dL. Atherogenic catalogues, with the TC/HDL ratio and the LDL/HDL ratio, were also determined to investigate the balance between protective and atherogenic lipoproteins:

$$\begin{aligned}\text{TC/HDL ratio} &= \text{TC}/\text{HDL} \\ \text{LDL/HDL ratio} &= \text{LDL}/\text{HDL}\end{aligned}$$

Determination of Oxidative Stress Markers and Antioxidant Enzymes

Oxidative stress markers and antioxidant defense parameters were evaluated using spectrophotometric techniques. Lipid peroxidation was measured by malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) method (Ohkawa et al., 1979), in which tissue homogenates were reacted with trichloroacetic acid and thiobarbituric acid, heated, centrifuged, and absorbance read at 535 nm; results were expressed as nmol MDA per mg protein. Superoxide dismutase (SOD) activity was assessed by its ability to prevent pyrogallol autoxidation, as described by Marklund and Marklund (1974), and results were expressed as units per mg protein. Catalase (CAT) activity was measured following Aebi (1984) by

monitoring the breakdown of hydrogen peroxide, with absorbance recorded at 405 nm and expressed as units per mg protein. Reduced glutathione (GSH) concentration was counted by means of Ellman's reagent (DTNB) method (Sedlak & Lindsay, 1968), and stated as $\mu\text{mol/g}$ tissue. Total antioxidant capacity (TAC) was determined by the ABTS radical cation decolorization assay and stated as mM Trolox equivalents (Re et al., 1999).

Determination of Immune and Inflammatory Markers

Serum levels of pro-inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), the anti-inflammatory cytokine interleukin-10 (IL-10), and C-reactive protein (CRP) were measured by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA) following the manufacturer's protocols. These assays have been extensively applied in experimental models to evaluate inflammatory and immune responses (Dinarello, 2000; Gabay & Kushner, 1999; Ahmed et al., 2025). Optical density was measured spectrophotometrically, and concentrations were calculated against standard curves created from known cytokine standards. TNF- α , IL-6, and IL-10 concentrations were stated in pg/mL , while CRP was reported in mg/L .

Statistical Analysis

One-way analysis of variance (ANOVA) was executed by using SPSS software (version 26.0) followed by Tukey's HSD post hoc test., to identify significant differences between the groups.

RESULTS

Table 2 presents the effects of MOS-NPs on the serum lipid profile and atherogenic indices in diet-induced hyperlipidemic Wistar rats. Dietary supplementation with MOS-NPs significantly improved the lipid profile of hyperlipidemic rats compared with the HC group. Rats fed a high-fat diet exhibited markedly elevated total cholesterol, triglycerides, LDL, and VLDL, along with reduced HDL levels relative to normal controls. MOS-NP treatment restored lipid parameters in a dose-dependent manner, with the high-dose group showing values approaching those of the normal control. Atherogenic indices, including the TC/HDL and LDL/HDL ratios, were significantly elevated in hyperlipidemic rats, whereas MOS-NP administration progressively reduced these ratios, indicating a strong protective effect against diet-induced dyslipidemia. Overall, MOS-NPs effectively modulated serum lipid disturbances caused by a high-fat diet.

Table 2. Effect of *Moringa oleifera* seed nanoparticles on serum lipid profile and atherogenic indices in diet-induced hyperlipidemic Wistar Rats

Group	NC	HC	MOS-NP-L	MOS-NP-M	MOS-NP-H	P value
TC (mg/dL)	114.3±3.8 ^a	217.5±8.4 ^c	177.8±6.3 ^b	152.4±4.6 ^b	128.5±4.4 ^{ab}	<0.001
TG (mg/dL)	83.3± 3.2 ^a	172.4±5.3 ^c	143.4±4.9 ^b	125.5±4.9 ^b	106.4±4.4 ^{ab}	<0.001
HDL (mg/dL)	56.5±2.4 ^a	28.3±1.3 ^c	38.4±1.7 ^b	45.4±2.2 ^b	53.4±2.4 ^a	<0.001
LDL (mg/dL)	38.1±1.6 ^a	144.2±5.7 ^c	109.6±4.5 ^b	81.4±4.3 ^b	59.4±1.5 ^{ab}	<0.001
VLDL (mg/dL)	17.4± 0.6 ^a	36.2± 1.6 ^c	28.8± 1.5 ^b	25.6± 1.3 ^b	21.3± 1.2 ^{ab}	<0.001
TC/HDL	2.04±0.08 ^a	7.42±0.33 ^c	4.31± 0.14 ^b	3.22± 0.16 ^b	2.44±0.14 ^{ab}	<0.001
LDL/HDL	0.66±0.04 ^a	4.92±0.22 ^c	2.75± 0.14 ^b	1.76± 0.06 ^b	1.14± 0.06 ^{ab}	<0.001

Note: Values are expressed as mean ± SE. Different superscript letters (a, b, c) within the same row designate significant differences among experimental groups (p < 0.05).

Table 3 summarizes the effects of *M. oleifera* seed nanoparticles (MOS-NPs) on oxidative stress indicators in diet-induced hyperlipidemic Wistar rats.

Table 3. Effect of *M. oleifera* seed nanoparticles on oxidative stress and antioxidant defense markers in diet-induced hyperlipidemic Wistar Rats

Group	NC	HC	MOS-NP-L	MOS-NP-M	MOS-NP-H	p-value
MDA (nmol/mg protein)	1.7± 0.4 ^a	5.3± 0.7 ^c	3.2± 0.6 ^b	2.5± 0.4 ^b	2.2± 0.4 ^{ab}	<0.001
SOD (U/mg protein)	64.7± 2.9 ^a	33.4± 1.6 ^c	46.2± 2.4 ^b	54.4 ± 2.4 ^b	60.7± 2.3 ^{ab}	<0.001
CAT (U/mg protein)	39.4± 1.4 ^a	20.1± 1.4 ^c	28.9± 1.4 ^b	34.2± 1.4 ^b	37.4± 1.3 ^{ab}	<0.001
GSH (μmol/g)	7.4 ± 0.4 ^a	3.6 ± 0.7 ^c	5.8± 0.1 ^b	6.2± 0.3 ^b	7.2± 0.4 ^{ab}	<0.001
TAC (mM Trolox eq.)	1.62± 0.06 ^a	0.74±0.04 ^c	1.03± 0.06 ^b	1.34± 0.06 ^b	1.59± 0.07 ^{ab}	<0.001

Note: Values are expressed as mean ± SE. Different superscript letters (a, b, c) within the same row designate significant differences among experimental groups (p < 0.05).

Rats fed a high-fat diet showed significant oxidative stress, with increased lipid peroxidation and markedly reduced antioxidant defenses compared with normal controls. MOS-NP supplementation improved oxidative status in a dose-dependent manner, reducing markers of lipid peroxidation while enhancing antioxidant enzyme activities and non-enzymatic antioxidants. The high-dose MOS-NP group restored oxidative stress parameters close to normal levels, indicating a strong protective effect against diet-induced oxidative damage.

Table 4 indicates that rats fed a high-fat diet showed marked immune dysregulation compared with the normal control group. Pro-inflammatory cytokines, TNF- α and IL-6, were significantly elevated, while the anti-inflammatory cytokine IL-10 was distinctly reduced. Similarly, CRP levels were substantially higher in the HC group. MOS-NP supplementation modulated these immune responses in a dose-dependent manner, lowering pro-inflammatory markers and enhancing anti-inflammatory cytokine levels. The high-dose MOS-NP group restored inflammatory markers to near-normal control levels, representing a strong immunomodulatory effect against diet-induced immune imbalance.

Table 4. Effect of *M. oleifera* seed nanoparticles on serum pro- and anti-inflammatory markers in diet-induced hyperlipidemic Wistar rats.

Group	NC	HC	MOS-NP-L	MOS-NP-M	MOS-NP-H	p-value
TNF-α (pg/mL)	24.4 $\pm$ 1.3 ^a	64.2 $\pm$ 2.9 ^c	49.4 $\pm$ 2.3 ^b	36.9 $\pm$ 1.7 ^b	28.6 $\pm$ 1.6 ^{ab}	<0.001
IL-6 (pg/mL)	31.4 $\pm$ 1.2 ^a	77.4 $\pm$ 3.4 ^c	58.6 $\pm$ 2.4 ^b	45.4 $\pm$ 2.4 ^b	34.2 $\pm$ 1.4 ^{ab}	<0.001
IL-10 (pg/mL)	45.6 $\pm$ 2.2 ^a	19.4 $\pm$ 1.2 ^c	28.2 $\pm$ 1.6 ^b	35.4 $\pm$ 1.6 ^b	42.1 $\pm$ 1.8 ^{ab}	<0.001
CRP (mg/L)	1.7 $\pm$ 0.2 ^a	6.2 $\pm$ 0.4 ^c	4.5 $\pm$ 0.3 ^b	3.2 $\pm$ 0.4 ^b	2.2 $\pm$ 0.6 ^{ab}	<0.001

Note: Values are expressed as mean $\pm$ SE. Different superscript letters (a, b, c) within the same row designate significant differences among experimental groups ($p < 0.05$).

DISCUSSION

The most significant improvement in serum lipid profiles was found in this study. Rats fed an HFD developed noticeable hyperlipidemia, as reflected by elevated serum TC, LDL, TG, and VLDL, along with reduced HDL. Treatment with MO-SeNPs markedly improved lipid parameters compared with the HFD group, with the high-dose treatment showing the highest enhancement, with values approaching those of the normal control group. MO-SeNP

supplementation reestablished these lipid parameters toward normal, with the highest dose showing effects analogous to those of standard lipid-lowering interventions reported in earlier studies (Xu et al., 2023; Mohammadi et al., 2024). Zakaria et al. (2023) described that *M. oleifera* seeds possess lipid-lowering properties by enhancing bile acid excretion and reducing intestinal cholesterol absorption. Ashfaq et al. (2023) and Gangavarapu et al. (2024) studied the nanoparticle formulation's progress in serum lipid profiles by facilitating targeted delivery, increasing bioavailability, consistent with reports of enhanced effectiveness of other phytochemical-loaded nanoparticles. Zhang et al. (2024) reported that hyperlipidemia is associated with dysregulation of lipid metabolism genes, viz., peroxisome proliferator-activated receptor alpha (PPAR- α) and sterol regulatory element-binding protein-1 (SREBP-1). They concluded that *M. oleifera* plant bioactives, such as flavonoids and isothiocyanates, upregulate PPAR- α and t and inhibit SREBP-1 to reduce lipid accumulation. Mastino et al. (2023) reported that inadequate antioxidant defenses and excessive production of reactive oxygen species led to oxidative stress and hyperlipidemia-mediated organ damage. In this study, HFD-fed rats showed higher MDA levels and reduced antioxidant enzyme activities, whereas MO-SeNP treatment significantly reduced MDA levels and reestablished antioxidant enzyme activities. These enhancements were particularly obvious in the treated groups compared with the HFD group, representing a protective antioxidant effect of MO-SeNP supplementation. These findings support previous studies showing that *M. oleifera* extracts reduce oxidative stress in cardiovascular models and in hepatic and renal systems (Mthiyane et al., 2022). Islam et al. (2023) reported an enhancement in antioxidant defenses in *M. oleifera* seeds, attributed to isothiocyanates and polyphenols, which directly upregulate antioxidant gene expression (Nrf2/ARE pathway) and scavenge ROS. In this study, HFD-fed rats showed marked improvements, and MO-SeNPs significantly exerted nephroprotective and hepatoprotective effects, with reduced levels of liver enzymes and renal biomarkers. Previous studies reported that *M. oleifera* seed and leaf extracts enhance mitochondrial function, stabilize cell membranes, reduce lipid peroxidation, and protect hepatocytes (Sabat et al., 2025). Similarly, the nephroprotective properties of *M. oleifera* were attributed to fewer glomeruli and oxidative damage in renal tubules (Hussein et al., 2024). The findings of this study also show that the nanoparticle form of *M. oleifera* seeds delivers improved protective outcomes, with improved delivery to target tissues. In this study, HFD-fed rats showed elevated TNF- α , IL-6, and CRP, and reduced IL-10. MO-SeNP treatment inverted this imbalance by upregulating IL-10 and downregulating pro-inflammatory markers. This modulation of inflammatory markers further supports the protective role of MOS-SeNP treatment against HFD-induced metabolic disturbances. These outcomes are consistent with those of Sailaja et al. (2021), who demonstrated that *M. oleifera*

isothiocyanates reduce the expression of inflammatory cytokines and inhibit NF- κ B activation. Karnwal et al. (2024) also reported that plant-derived nanoparticles enhance anti-inflammatory efficacy by promoting sustained release and increased tissue saturation. Therefore, in this study with MO-SeNPs, immunomodulatory effects were detected that could break the vicious cycle of inflammation and oxidative stress, which drives hyperlipidemia-induced organ damage.

CONCLUSION

This investigation concluded that *M. oleifera* seed nanoparticles effectively counteract hyperlipidemia-induced complications in rats through multiple mechanisms. This study confirms significant improvements compared with the HFD group, and some high-dose parameters advanced, but NC values did not increase to the same extent. Dose-dependent improvements were observed in lipid profiles, antioxidant capacity, and inflammatory markers, with the highest dose (200 mg/kg) restoring parameters to near-normal levels. These results indicate therapeutic effects while reflecting minor disparities in some parameters. The nanoformulation's superior efficacy over conventional extracts suggests enhanced bioavailability and targeted delivery. These findings support the therapeutic potential of MO-SeNPs as a natural intervention for managing metabolic disorders associated with hyperlipidemia. Further clinical studies are warranted to validate these protective effects in human subjects.

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