Received: 2 March 2022 | Revised: 16 May 2022 | Accepted: 18 May 2022

DOI: 10.1111/jfbc.14265

Journal of Food Biochemistry WILEY

ORIGINAL ARTICLE

Evaluating anti-obesity potential, active components, and antioxidant mechanisms of *Moringa peregrina* seeds extract on high-fat diet-induced obesity

Naglaa Elarabany[1,2] | Abeer Hamad[1,3] | Sanad M. AlSobeai[1]

[1]Biology Department, Sajir College of Arts and Science, Shaqra University, Shaqra, Saudi Arabia

[2]Zoology Department, Faculty of Science, Damietta University, Damietta, Egypt

[3]Biology Department, College of Applied and Industrial Science, Bahri University, Khartoum, Sudan

Correspondence
Naglaa Elarabany, Biology Department, Sajir College of Arts and Science, Shaqra University, Shaqra, Saudi Arabia.
Email: elarabny@gmail.com

Abstract

There are no medical drugs that provide an acceptable weight loss with minimal adverse effects. This study evaluated the *Moringa peregrina* (MP) seed extract's anti-obesity effect. Twenty-four (6/each group) male Sprague Dawley rats were divided into group I (control), group II (high-fat diet [HFD]), group III (HFD+ MP [250 mg/kg b.wt]), and group IV (HFD+ MP [500 mg/kg b.wt]). MP administration significantly ameliorated body weight gains and HFD induced elevation in cholesterol, triglycerides, LDL, and reduced HDL. Moreover, MP seed oil showed high free radical-scavenging activity, delayed β-carotene bleaching and inhibited lipoprotein and pancreatic lipase enzymes. High-performance liquid chromatography (HPLC) revealed three major active components: crypto-chlorogenic acid, isoquercetin, and astragalin. Both quantitative Real-time PCR (RT-PCR) and western blotting revealed that MP seeds oil significantly decreased the expression of lipogenesis-associated genes such as peroxisome proliferator-activated receptors gamma (PPARγ) and fatty acid synthase (FAS) and significantly elevated the expression of lipolysis-associated genes (acetyl-CoA carboxylase1, ACCl). The oil also enhanced phosphorylation of AMP-activated protein kinase alpha (AMPK-α) and suppressed CCAAT/enhancer-binding protein β (C/EBPβ). In conclusion, administration of *M. peregrina* seeds oil has anti-obesity potential in HFD-induced obesity in rats.

Practical applications

M. peregrina seeds oil had a potential anti-obesity activity that may be attributed to different mechanisms. These included decreasing body weight, and body mass index and improving lipid levels by decreasing total cholesterol, triglycerides and LDL-C, and increasing HDL-C. Also, *M. peregrina* seeds oil regulated adipogenesis-associated genes, such as downregulating the expression of (PPARγ, C/EBPα, and FAS) and improving and upregulating the expression and phosphorylation of AMPKα and ACCl. Despite that *M. peregrina* extract has reported clear anti-obesity potential through animal and laboratory studies, the available evidence-based on human clinical trials are very limited. Therefore, further studies are needed that could focus on clinical trials investigating anti-obesity potential different mechanisms of *M. peregrina* extract in humans.

KEYWORDS
anti-obesity, antioxidant mechanisms, *Moringa peregrina*, seeds oil

1 | INTRODUCTION

In the last four decades, obesity worldwide rates have tripled and are constantly elevating in pandemic areas (Kinnunen et al., 2021). Thus, it represents a global public health issue (Kinnunen et al., 2021). Obesity and overweight are serious risk factors for many non-communicable disorders including musculoskeletal, kidney and cardiovascular diseases, some cancers, atherosclerosis, type 2 diabetes, and other chronic disorders (Nyberg et al., 2018). Elevated body mass index (BMI) is the fourth leading cause of risk-associated mortality, with a declined life expectancy of 5–20 years, depending on comorbidities and condition severity (Kinnunen et al., 2021). Moreover, global health costs related to obesity and its complications are estimated to be about 2 trillion US$ (Fan et al., 2021). If obesity prevalence remains on its elevating trend, almost half of the world's adult population could be obese or overweight by 2030, imposing even greater economic, social, and personal costs (Fan et al., 2021).

There are several ways to reduce weight, including pharmacological agents; however, some of them have withdrawn from the market for safety concerns owing to their side effects (Onakpoya et al., 2016). Other strategies were used, such as reducing energy intake, rising physical activity, and increasing polyphenols-rich food consumption (lead to great satiety and fat oxidation as well as energy expenditure) (Kinnunen et al., 2021). There are almost no pharmacological therapies that give continuous weight loss with negligible unfavorable side effects (Khera et al., 2016). So, many efforts have been done to reduce body weight using pharmacological treatments that possess minimal adverse effects (Xie et al., 2021). In handling several chronic conditions owing to their efficacy, herbal supplements are being vastly used (Fan et al., 2021). When compared to chemically synthesized drugs, they have minimum/no toxic side effects and are cheap (Kinnunen et al., 2021). Herbals cover several natural products, including plant crude extracts and isolated compounds (Atanasov et al., 2021).

One of the most widely naturalized and distributed monogeneric family species is *Moringa peregrina* which has several nutritional and medicinal values related to its fruits, seeds, leaves, flowers, roots, and bark (Islam et al., 2021). *M. peregrina* like other Moringa family contains terpenoids, glycosides flavonoids, tannins, and saponins, all of which possess medicinal benefits (Islam et al., 2021). These compounds also possess effective antimicrobial, anti-carcinogenic, and antioxidant potential, and thus they can aid the immune system (Islam et al., 2021). These effective roles are mediated through decomposing peroxides, free radicals neutralization, or quenching singlet or triplet oxygens (Islam et al., 2021). In addition, tannins and saponins have been related to reduced energy needs for lipid and protein biosynthesis, leading to growth performance, nutrient utilization, energy retention, and declined body lipid (Dongmeza et al., 2006). Thus, such components have been reported to have efficient anti-obesity potential (Islam et al., 2021).

This study aimed to evaluate the potential of *M. peregrina* seeds extract to control obesity. This aim was obtained by determining body weight, body mass index (BMI), lipid profile, different antioxidant assays, proteins, and genes expression associated with lipogenesis and lipolysis in male Sprague Dawley rats.

2 | MATERIAL AND METHODS

2.1 | Plant material

Seeds of *M. peregrina* were obtained from a local market in Riyadh, KSA. Then, 500 g of uncoated seeds were dried by air and turned to powder using a scientific blender. Methanol was used for extraction by soaking 20 g of seeds crushed with 1 L of solvent and left overnight. After evaporating the solvent, the remaining plant material was further extracted twice with the same solvent to maximize the yield. Oil was purified by Soxhlet apparatus which was applied for oil extraction by dichloromethane/methanol (1:1, v/v) mixture (Abd El Baky & El-Baroty, 2013; Cequier-Sánchez et al., 2008).

2.2 | High-performance liquid chromatography (HPLC)

On reverse-phase Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm), HPLC (Agilent 1000) analysis of *M. peregrina* extract phenolic compounds was performed. According to Öztürk et al. (2007), a gradient program is used including two solvents system (A: acetic acid (0.5% in acetonitrile)/water (1/1) B: acetic acid (2% in water)) at a constant solvent flow rate of 1.2 ml/minute. The injection volume was 20 μl. By UV–VIS detection, signals were detected at 280 nm. All solutions were prepared with deionized water.

2.3 | Animals

Twenty-four male Sprague Dawley rats (110–120 g) were included and kept in Laboratory Animal House, King Saud University, Riyadh, Saudi Arabia. Randomly, they were assigned into four groups, with six rats per group. For acclimation and adaptation, rats were fed ad libitum control diets formulated according to National Research Council (1995) (see Table 1 for ingredient composition) for 2 weeks, maintained under natural daylight rhythm and room temperature. According to Axen and Axen (2006) and Kilany et al. (2020), obesity

TABLE 1 Composition of experimental diets

Ingredients	Control diet (%)	High-fat diet (%)
Yellow corn	70.24	29.50
Corn gluten	5.00	2.1
Soybean	8.80	3.70
Casein	5.00	13.84
Wheat bran	4.00	1.68
Sucrose	–	5.14
Vegetable oil	4.74	1.99
Animal fat	–	38.20
Cellulose	–	1.50
Methionine	0.34	
Lysine	0.07	0.39
Ground limestone	0.82	–
Dicalcium phosphate	0.56	0.42
Common salt		1.11
Premix	0.13	0.13
	0.30	0.30
Total	100.00	100.00
Calculated values		
CP %	17.21	17.20
ME (Kcal/kg)	3362.80	5001.75
Ca	0.50	0.50
P	0.30	0.30

was induced in rats by feeding HFD (see Table 1 for ingredient composition) for 12 weeks. The study design was approved by the ethical committee of King Saud University.

2.4 | Animals groups

Animals were divided into four groups as follows:

Group I (control): received a control diet during the whole experimental period.

Group II (negative control): received HFD all over the experimental period.

Group III (Dose 1): received HFD all over the 12 weeks and *M. peregrina* seeds oil (250 mg/kg b.wt/day) by intragastric administration for the last 8 weeks.

Group IV (Dose 2): received HFD all over the 12 weeks and *M. peregrina* seeds oil (500 mg/kg b.wt/day) by intragastric administration for the last 8 weeks.

The MP doses used in this experiment was chosen according to previous reports where doses 300 to 600 mg/kg MP produced anti-obesity and anti-hyperlipidemic effects on high-fat-diet-fed rats (Alkhudhayri et al., 2021). Moreover, *Moringa oleifera* was shown to exhibit potent hypolipidemic and anti-obesity effects in a similar animal model at doses of 200, 400, and 600 mg/kg (Bais et al., 2014; Metwally et al., 2017).

2.5 | Body weight, blood sampling, and biochemical parameters

To obtain weight gain, final body weights were compared to initial weights for each rat. From each rat after overnight fasting, under the effect of diethyl-ether anesthesia, a *retro*-orbital venous blood sample was drawn and collected in a plain tube to separate sera. Serum levels of high-density lipoprotein cholesterol (HDL-C), triglycerides, and total cholesterol were determined by Spinreact, Spain kits. By the formula of Friedewald et al. (1972), low-density lipoprotein cholesterol (LDL-C) was determined.

$$\text{Serum LDL} - \text{C (mg / dl)} = \text{TC} - \text{HDL} - \text{C} - \frac{\text{TGs}}{5}$$

After blood samples, all rats were euthanized by an overdose of diethyl-ether. Adipose tissue from mesenteric, perirenal, and epididymal sites were dissected out, rinsed with phosphate buffer solution and weighed immediately then stored in −80 until the beginning of analysis.

2.6 | 2,2′-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay

M. peregrina seeds oil radical scavenging activity against DPPH radical was determined according to a previous method described by Zhu et al. (2015). In a 96-well plate, 10 µl of positive control solutions (31.25–1000 µM, Trolox and Ascorbic acid) or adequate diluted samples were mixed with 190 µl of methanol solution of DPPH (0.1 mM). After 30 min of reaction in darkness at room temperature, reaction mixture absorbance was detected at 517 nm with a microplate reader (Tecan Infinite M200 PRO, TECAN, Männedorf, Switzerland). At the same time in this assay, methanol was used as a blank control, and all controls and extracts were screened in triplicate. DPPH inhibition percentage was computed and measured using the following equation: Inhibition rate (%) = $[(Ab_C - Ab_S)/Ab_C] \times 100$, where Ab_C is the blank control absorbance value, Ab_s is the tested sample absorbance value, and the IC_{50} value was obtained when DPPH free radicals were inhibited by 50%.

2.7 | 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay

M. peregrina seeds oil ABTS radical scavenging activity was determined according to a previous method by Zhu et al. (2015). Working $ABTS^+$ (radical cation) (Sigma-Aldrich, USA) solution was prepared by mixing potassium persulfate (4.9 mM in H_2O) and ABTS solution (7 mM in H_2O) in equal quantities, and allowed them to react, for 12–16 h, in darkness at room temperature. Appropriately, $ABTS^+$ solution was diluted with methanol to achieve an absorbance value at 734 nm of 0.70 ± 0.10. After that in a 96-well plate, the mixture solution consisting of appropriately diluted samples (10 µl) or Vitamin C

solution (31.25–1000 μM) and $ABTS^+$ solution (190 μl) was incubated in darkness for 30 min before reading the absorbance at 734 nm. Vitamin C was used as positive control and methanol as blank. All controls and extracts were tested in triplicate. ABTS assay IC_{50} value was calculated at different concentrations using the same formula described in the DPPH assay.

2.8 | Pancreatic lipase (PL) inhibitory assay

M. peregrina seed oil's ability to inhibit PL was determined using p-NPB (P-nitrophenyl butyrate) as the substrate according to Kim et al. (2007). Enzyme buffer was obtained by adding PL (10 units, 30 μl) in 1 Mm EDTA (pH 6.8), 10 mM MOPS and 850 μl of Tris-buffer (pH 7.0; 100 mM Tris-HCl, 5 mM CaCl2). With 880 μl of enzyme buffer, the test sample (100 μl) was mixed and incubated at 37°C for 15 min. Then, the substrate solution (20 μl; 10 mM p-NPB) was added and the enzymatic reaction was allowed at 37°C for 15 min. By measuring P-NPB hydrolysis to P-Nitrophenol at 405 nm, the lipase activity was measured using an ELISA reader. Lipase inhibition = 1 (Absorbance of sample × 100)/Control.

2.9 | β-carotene-linoleic acid activity

β-carotene and linoleic acid oxidation inhibition were measured according to a previously described method by Ndhlala et al. (2014). In brown Schott bottle, 10 mg β-carotene was dissolved in chloroform (10 ml). Under vacuum, excess chloroform was evaporated resulting in β-carotene thin film near to dryness. Immediately to this thin film, 200 μl of both linoleic acid and Tween 20 were added and mixed with 497.8 ml distilled water, giving the final β-carotene concentration (20 μg/ml). By vigorous agitation, this solution was further saturated with oxygen to form an orange-colored emulsion. Into test tubes, 200 μl of *M. peregrina* seeds oil or butylated hydroxytoluene (6.25 mg/ml) were dispensed to this emulsion (4.8 ml), giving a final reaction mixtures concentration (250 μg/ml). At 470 nm, absorbance for each reaction was immediately ($t = 0$) determined. Then after incubation at 50°C, absorbance was measured every 30 min for 180 min. Instead of the sample, 50% methanol was used as a negative control. β-carotene bleaching rate was calculated using the following formula: rate of bleaching (R)= $\{ \ln (At = 0) / (At = t) \} \times 1 / t$, Where: $At = t$ is the absorbance at time 90 min and $At = 0$ is the absorbance of the emulsion at 0 min. Average rates were used to measured antioxidant activity (ANT), and expressed as inhibition percent of β-carotene bleaching rate: % ANT = ((R control-R sample)/(R control)) × 100.

2.10 | Lipoprotein lipase (LPL) assay

In 15-ml glass test tubes, diluted (25 units; 0.5 ml) LPL (Sigma-Aldrich, USA) and 0.5 ml of each *M. peregrina* extract concentration were mixed and preincubated for 30 min at 4°C. Then to initiate hydrolysis, 1 ml of prepared substrate emulsion was added to the mixture and incubated in a water bath at 37°C. After 30 min, the reaction was stopped by NaCl (2.0 ml, 1 M, Sigma-Aldrich). Concurrently, controls were carried out with enzymes mixture and substrate only, without plant extracts. Liberated free fatty acids were then titrated with standardized NaOH (0.01 M) to pH 9.2. The liberated free fatty acid amount was calculated from the amount of base added as previously described (Chung & Scanu, 1974).

2.11 | Western blotting

The total protein was extracted from adipose tissues using the Tri-Fasted kit (Peqlab, VWR Company, USA). According to Bradford method (Bradford, 1976) using a reference range (0–150 μg) of bovine serum albumin (BSA), the protein concentration was determined based on the interaction between protein and Coomassie Brilliant Blue G250 (CBBG-250) in acidic conditions. Extracted proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred onto Hybond™ nylon membrane (GE Healthcare Biosciences, UK). With 5% BSA, each membrane was blocked for 30 min. Then, membranes were incubated overnight separately at 4°C with primary antibodies [AMPK α (SAB4503754, Sigma Aldrich, USA), AMPK (A 769662, Creative Biolabs), Anti-C/EBP α Antibody (sc-365,318 Santa Cruz Biotechnology, CA, USA), and β-actin (1:2000, ORIGENE, USA)]. The next day using PBS plus Tween 20, membranes were washed five times. Then, membranes were incubated for 1 h at room temperature with goat anti-mouse/anti-rabbit secondary antibody conjugated to horseradish peroxidase (0.1–0.5 μg/ml, R&D Systems, USA) before they were washed five times with PBS plus Tween 20. Finally, protein bands were visualized and quantified with GraphPad Prism software (San Diego, CA, USA).

2.12 | Quantitative gene expression using real-time PCR (qRT-PCR)

Total RNA was extracted from adipose tissues according to kit (TRIzols, 15,596,026, Life Technologies, USA) instructions. Extracted RNA (1 μg) was used to synthesize cDNA (Qiagen, USA), which then was amplified using SYBR Green master mix (Thermo Scientific, USA) for peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACCl). The primer sequences for studied genes are presented in Table 2. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method in the CFX96 Touch™ (Bio-Rad Co., USA).

2.13 | Statistical analysis

Statistical analysis was performed using version 20 SPSS (SPSS Inc., Chicago). Variables were represented as mean ± *SD*. The *ANOVA*

TABLE 2 Primer sequences of the studied genes

Target gene	Probe	Reference
ACC1	GATGAACCATCTCCGTTGGC GACCCAATTATGAATCGGGAGTG	Zhou et al. (2021)
Fatty acid synthase (FAS) gene	AAGGACCTGTCTAGGTTTGATGC TGGCTTCATAGGTGACTTCCA	Barbagallo et al. (2016)
Peroxisome proliferator-activated receptor γ (PPARγ)	ACCAAAGTGCAATCAAAGTGGA ATGAGGGAGTTGGAAGGCTCT	

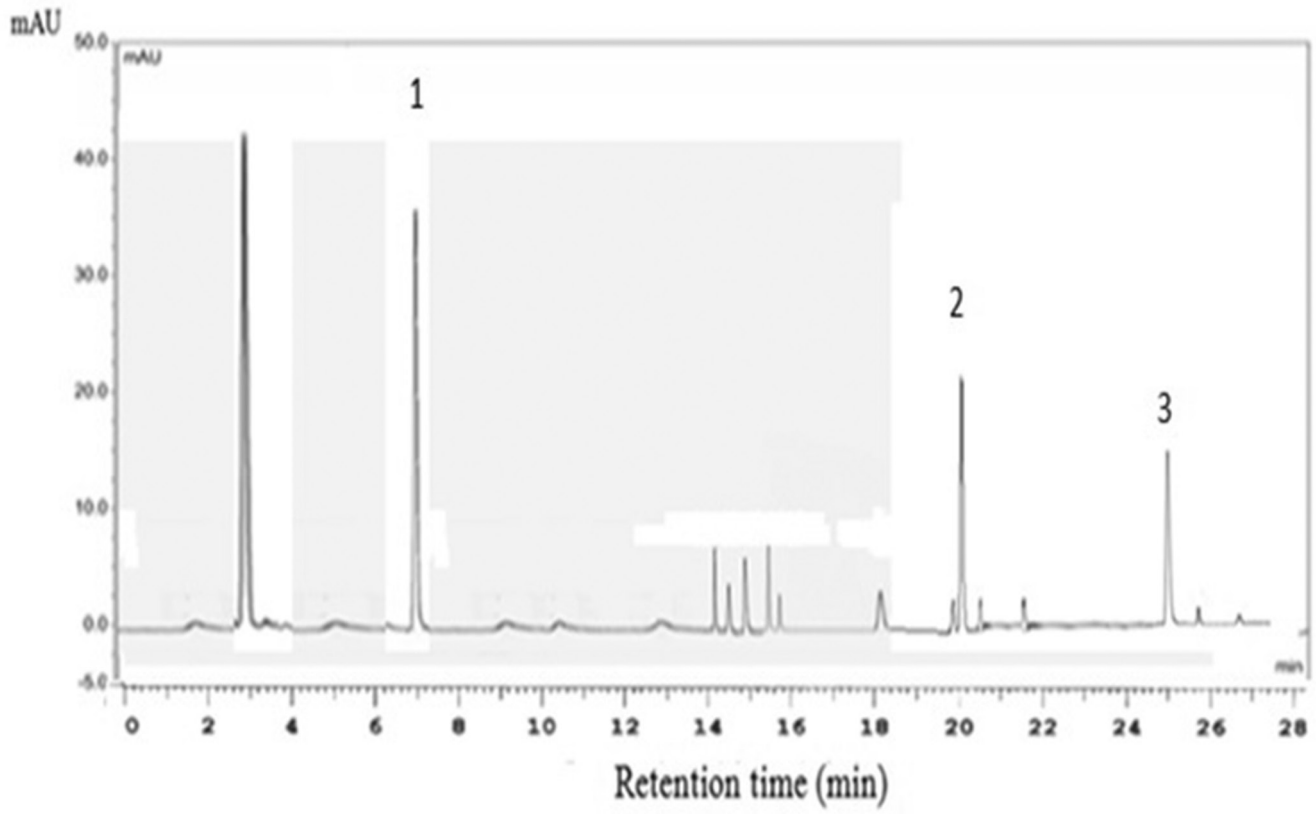

FIGURE 1 HPLC chromatograms of *M. peregrina* seeds extract constituents. Peaks 1, 2, and 3 were corresponding to crypto-chlorogenic acid, isoquercetin, and astragalin amino acid.

test followed by Duncan's test was used for different assessments. $p < .05$ was statistically significant.

3 | RESULTS

3.1 | HPLC analysis

HPLC (Figure 1) was used for qualitative and quantitative analysis of *M. peregrina* seeds extract. It revealed three major peaks corresponding to crypto-chlorogenic acid (at 7.01 min, concentration = 36.61 μmoL/L), isoquercetin (at 19.91 min, concentration = 20.11 μmoL/L), and astragalin (at 25.1 min, concentration = 14.48 μmoL/L).

3.2 | Body weight, BMI, and lipid profile

After obesity induction, rats' body weight and body mass index in group II significantly ($p < .05$) increased in control (group I). *M. peregrina* seeds oil administration in both groups III (250 mg/kg b.wt) and IV (500 mg/kg b.wt) significantly decreased final body weights and body mass index as compared to group II ($p < .05$, Table 3). Compared to the control group, HFD was reported to significantly enhance obesity induction ($p < .05$, group II) with elevation in cholesterol, triglycerides, and LDL-C levels. Rats treated with *M. peregrina* seeds oil significantly decreased the former biochemical parameters than of group II ($p < .05$), and sometimes their levels were comparable to controls. In contrast, HDL-C levels significantly dropped after HFD administration in group II ($p < .05$) and *M. peregrina* seeds oil

TABLE 3 Effect of high-fat diets and *M. peregrina* seeds oil extract on body weight and lipid profile

Parameter	Group I Control	Group II Negative control	Group III MP dose 1 (250 mg/kg)	Group IV MP dose 2 (500 mg/kg)
Initial body weight (g)	110.8 ± 6.3^{a}	112.6 ± 6.9^{a}	109.8 ± 7.3^{a}	110.5 ± 6.9^{a}
Final body weight (g)	222.3 ± 18.5^{a}	260.5 ± 19.6^{b}	215.6 ± 10.1^{ac}	210.5 ± 10^{ac}
Body mass index (BMI)	0.4 ± 0.01^{a}	0.48 ± 0.01^{b}	0.37 ± 0.01^{ac}	0.37 ± 0.01^{ac}
Cholesterol (mg/dl)	211.6 ± 11.2^{a}	251.7 ± 10.2^{b}	210.6 ± 9.2^{ac}	205.2 ± 12.2^{ac}
Triglycerides (mg/dl)	129.6 ± 10.2^{a}	191.4 ± 10.2^{b}	135.6 ± 10.2^{ac}	129.2 ± 15.2^{ac}
LDL-C (mg/dl)	140.5 ± 9.2^{a}	170.7 ± 10.2^{b}	145.9 ± 8.2^{ac}	143.5 ± 11.2^{ac}
HDL-C (mg/dl)	41.5 ± 8.2^{a}	36.4 ± 9.2^{b}	40.6 ± 10.2^{ac}	41.7 ± 15.2^{ac}

Note: Different superscripts ^{a,b,c} within the same row represent significance at $p < .05$.
Abbreviations: HDL-C, -densityhigh lipoprotein cholesterol; LDL-C, -densitylow lipoprotein cholesterol.

TABLE 4 Corresponding antioxidant and anti-obesity activity of *M. peregrina seeds oil*

Parameter	*M. peregrina* (250 mg/kg)	*M. peregrina* (500 mg/kg)
DPPH IC_{50} (μg/ml)	29.41 ± 1.1	22.64 ± 0.8
DPPH activity (%)	62.64 ± 3.0	81.52 ± 2.9
ABTS IC50 (μg/ml)	93.3 ± 5.4	89.7 ± 4.2
ABTS activity (%)	72.62 ± 3.2	88.95 ± 3.1
β-carotene bleaching IC50 (μg/ml)	59.81 ± 4.0	72.54 ± 3.5
β-carotene bleaching activity (%)	21.65 ± 1.2	44.8 ± 2.2
Pancreatic lipase IC50 (μg/ml)	55.14 ± 4.0	34.84 ± 2.9
Pancreatic lipase activity (%)	21.7 ± 2.0	39.5 ± 2.7
Lipoprotein lipase IC50 (μg/ml)	47.82 ± 3.1	77.8 ± 4.0
Lipoprotein lipase inhibition (%)	23.66 ± 2.4	51.80 ± 4.1

Notes: Results were expressed as mean ± standard deviation; DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); When radicals of DPPH and ABTS were inhibited by 50%, the half-maximal inhibitory level (IC_{50}) was obtained.

administration significantly raised these levels compared to group II ($p < .05$, Table 3).

3.3 | *M. peregrina* seeds oil extract antioxidant and anti-obesity activities

In this study, we used different assays (DPPH, ABTS, β-carotene bleaching, Pancreatic, and Lipoprotein lipases assays) to assess and compare the anti-obesity and antioxidant potentials of *M. peregrina* seeds oil. In the case of 250 mg/kg b.wt dose, IC_{50} values of *M. peregrina* seeds oil extract were 29.41 ± 1.1, 93.3 ± 5.4, 59.81 ± 4.0, 55.14 ± 4.0, and 47.82 ± 3.1 for DPPH, ABTS, β-carotene bleaching, pancreatic, and lipoprotein lipases assays, respectively. Dose 500 mg/kg b.wt was more active and had IC_{50} values of 22.64 ± 0.8, 89.7 ± 4.2, 72.54 ± 3.5, 34.84 ± 2.9, and 77.8 ± 4.0, respectively (Table 4).

3.4 | *M. peregrina* seeds oil inhibits lipogenesis and activates AMPK signaling

Western blotting for AMPK and AMPKα proteins was used to investigate whether *M. peregrina* seeds oil has an inhibitory effect on lipogenesis due to AMPK activation or not. Results revealed that *M. peregrina* seeds oil significantly increased the expression of both AMPK (Figure 2a) and AMPKα (Figure 2b) proteins in a dose-dependent manner. Moreover, *M. peregrina* seeds oil significantly decreased the expression of the lipogenesis-associated protein (C/EBPα) (Figure 2c). Interestingly, real-time PCR also revealed that *M. peregrina* seeds oil significantly decreased the expression of lipogenesis-associated genes (PPARγ and FAS) and significantly elevated the expression of the lipolysis-associated genes ACCI (Table 5).

4 | DISCUSSION

Obesity is a condition that is associated with many chronic complications development, such as hormonal imbalance, dyslipidemia, impaired glucose tolerance, and hyperglycemia and, thus, it is the main risk for elevated mortality and morbidity (Ali Redha et al., 2021). There are no medical drugs or pharmacological management that provide an acceptable weight loss with minimal adverse effects (Müller et al., 2021). Previous studies regarding the lethal dose (LD_{50}) value of some *M.* species reported that its extract was safe and nontoxic up to 5 g/kg body weight (Bais et al., 2014). Some *M.* family members such as *M. oleifera* were reported to have wound healing, antifertility, anti-inflammatory, diuretic, antiulcer, and anti-obesity properties (Ali Redha et al., 2021). But, still, limited pieces of evidence are available for *M. peregrina's* anti-obesity potential. Hence, the study has been aimed to demonstrate the effect of *M. peregrina* seeds oil in high-fat-diet-induced obesity.

In this study, weight gain and relative fat mass were increased in the HFD group compared to the control group. Also, HFD stimulated the elevation in cholesterol, triglycerides, and LDL-C levels.

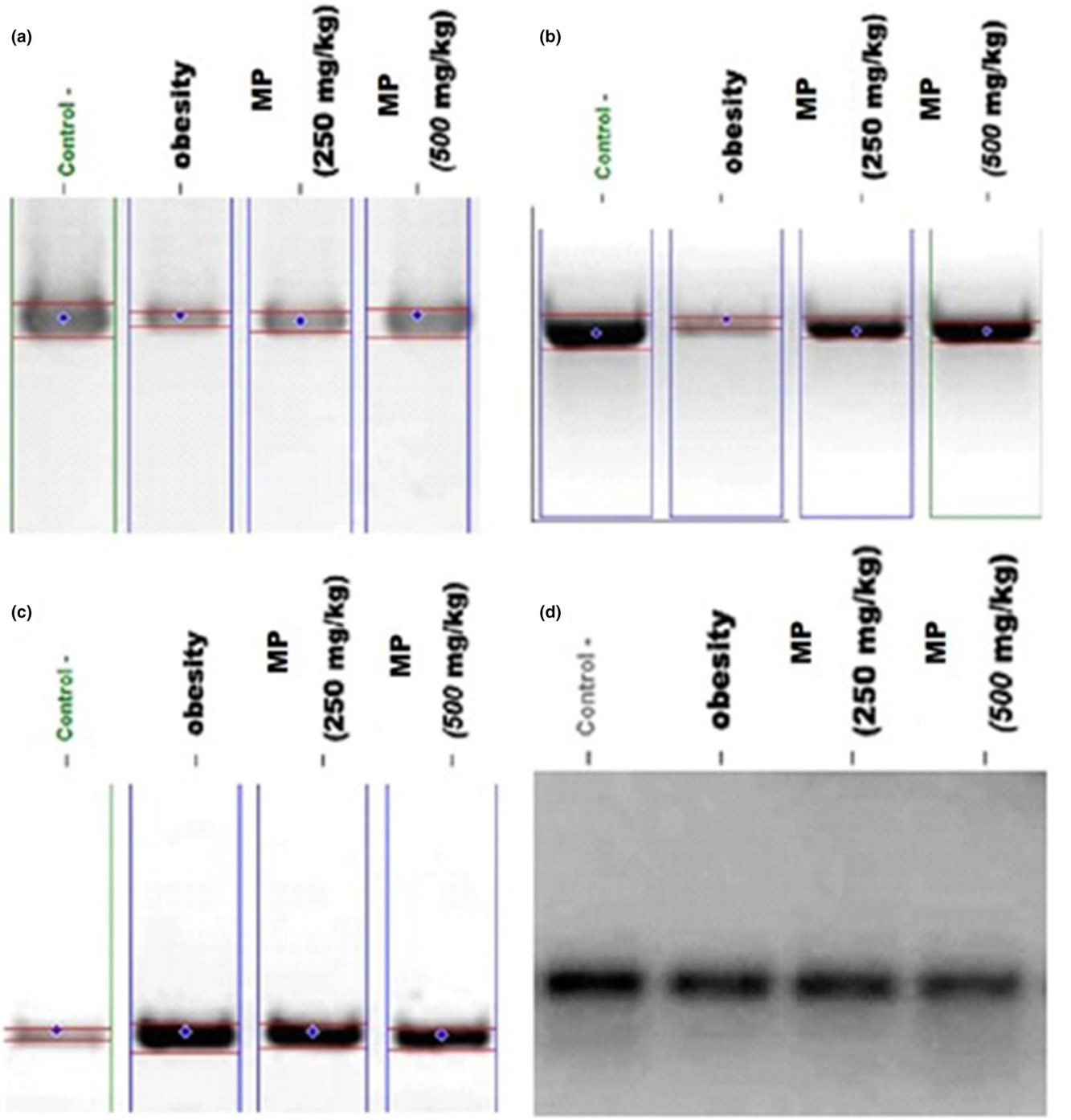

FIGURE 2 *M. peregrina* (MP) seeds extract inhibits lipogenesis and activates AMPK signaling. In a dose-dependent manner, treatment with *M. peregrina* seeds extract significantly activates the expression of both (a) AMPK and (b) AMPKα proteins. (c) it inhibits lipogenesis-associated protein (C/EBPα). Each value was normalized to (d) β-actin expression.

In contrast, HDL-C levels were significantly ($p < .05$) dropped after HFD administration. These results were in agreement with Kostrycki et al. (2019) and may be attributed to higher energy intake in the HFD group that leads to weight gain consequent to the elevated fat mass. Treatment with *M. peregrina* seeds oil extract revealed a significant reduction in body weight, cholesterol, triglycerides, and LDL-C and a significantincrease in HCL-C that was in harmony with previous findings such as these of Kilany et al. (2020), Bais et al. (2014), and Nahar et al. (2016), and similar results were achieved with the administration of MP leaves extract (Alkhudhayri et al., 2021).

These findings suggested the hypolipidemic effect of *M. peregrina* seeds oil extract as well as its ability to reduce fat mass and body weight.

TABLE 5 Analysis of gene expression by real-time PCR of PPARγ, FAS, and ACCI

Genes	Groups	Tested experimental gene	Tested control gene	Housekeeping gene experimental	Housekeeping gene control	ΔCt values for the experimental	ΔCt values for the control	Delta Ct value (ΔΔCt)	2^-ΔΔCt (relative gene expression)
PPARγ	I	29.8 ± 0.15	22	27.6 ± 0.09	21.3	2.2 ± 0.1	0.7	1.5 ± 0.1	0.4 (0.3–0.6)^{a}
	II	29.5 ± 0.06	22	29 ± 0.08	21.3	0.5 ± 0.1	0.7	−0.2 ± 0.1	1.1 (0.9–1.3)^{b}
	III	29.5 ± 0.07	22	28.6 ± 0.07	21.3	0.9 ± 0.1	0.7	0.2 ± 0.1	0.9 (0.8–1.1)^{ac}
	IV	29.3 ± 0.07	22	28 ± 0.07	21.3	1.3 ± 0.1	0.7	0.6 ± 0.1	0.7 (0.5–1.3)^{ac}
FAS	I	31.9 ± 0.17	21.9	29.9 ± 0.09	20.7	2 ± 0.1	1.2	0.8 ± 0.1	0.6 (0.5–1.2)^{a}
	II	31 ± 0.15	21.9	30.5 ± 0.09	20.7	0.5 ± 0.08	1.2	−0.7 ± 0.08	1.6 (1.4–1.9)^{b}
	III	32 ± 0.15	21.9	31.2 ± 0.1	20.7	0.8 ± 0.1	1.2	−0.4 ± 0.1	1.3 (1.1–1.8)^{ac}
	IV	32.5 ± 0.18	21.9	31.2 ± 0.1	20.7	1.3 ± 0.1	1.2	0.1 ± 0.1	0.9 (0.8–1.1)^{ac}
ACC1	I	24.9 ± 0.03	20.7	24 ± 0.06	19.8	0.9 ± 0.09	0.9	0 ± 0.09	1.0 (0.9–1.1)^{a}
	II	25.6 ± 0.07	20.7	23.1 ± 0.05	19.8	2.5 ± 0.1	0.9	1.6 ± 0.1	0.3 (0.2–0.5)^{b}
	III	25.1 ± 0.07	20.7	23.3 ± 0.04	19.8	1.8 ± 0.1	0.9	0.9 ± 0.1	0.5 (0.4–0.7)^{ac}
	IV	25 ± 0.06	20.7	23.8 ± 0.06	19.8	1.2 ± 0.1	0.9	0.3 ± 0.1	0.8 (0.6–1.1)^{ac}

Note: Different superscripts ^{a,b,c} represent significance at $p < .05$.

Abbreviations: ACCI, acetyl-CoA carboxylase; FAS, fatty acid synthase; PPARγ, peroxisome proliferator-activated receptor γ.

M. peregrina seeds oil extract administration perfectly ameliorated oxidative stress induced by obesity and promoted antioxidant reserve (Kilany et al., 2020). Because of natural phytochemicals' complexity and their different modes of action, it is imprecise to determine the antioxidant potential by only a single method (Xu et al., 2019). Thus, in this study, we used different assays (DPPH, ABTS, β-carotene bleaching, pancreatic, and lipoprotein lipases) to assess the anti-obesity and antioxidant potentials of *M. peregrina* seeds oil. In a dose-dependent manner, *M. peregrina* seeds oil showed high DPPH and ABTS radical-scavenging activity. Also, its antioxidant activity was reported in the light of delayed β-carotene bleaching and inhibiting lipases enzymes.

M. peregrina's antioxidant effects may be associated with its great content of various natural antioxidant types such as ascorbic acid, β-sitosterol, vitamin A, phenolic compounds, glycosides, terpenoids, flavonoids, saponins, tannins, and β-carotene (Anwar et al., 2007; Lopez-Teros et al., 2017). Compared to a single antioxidant, these antioxidants combination in *M. oleifera* has been reported to have more efficacy owing to elevated antioxidant cascade mechanisms and their synergistic mechanisms (Tejas et al., 2012). Due to their ability to prevent free radicals, from hydroperoxides decomposition, and formation, phenolic compounds act as primary antioxidants (Kilany et al., 2020). These compounds constitute the main cornerstone in the decomposition of peroxides, neutralizing free radicals, and quenching singlet/triplet oxygen (Kilany et al., 2020).

Crypto-chlorogenic acid, isoquercetin, and astragalin were reported to be major active components in *M.* extracts (Vongsak et al., 2013). Here, HPLC analysis revealed that these amino acids were the pure active components of *M. peregrina* seeds oil extract. These acids are powerful natural antioxidant agents that possess several potential pharmacological activities including inflammation attenuation, dermatitis inhibition, antihypertension, cellular protective effect, anti-obesity, and reduction of organ and plasma lipids (Vongsak et al., 2013).

Adipogenesis is regulated by several genes including PPARγ (Ebrahimi et al., 2020). Also, PPARγ regulates lipogenesis and adipogenesis by inducing some enzymes activity such as FAS and ACC involved in adipocyte differentiation and lipid synthesis (Ebrahimi et al., 2020). Here, real-time PCR revealed that *M. peregrina* seeds extract significantly decreased the expression of lipogenesis-associated genes (PPARγ and FAS) and significantly elevated the expression of ACCI.

Adipogenesis and adipocyte differentiation are the main reasons for obesity (Sethi & Vidal-Puig, 2007). It is regulated by the expression of many transcription factors such as C/EBPα, which are activated at different stages during the adipocyte differentiation process to regulate genes expression in adipocytes, and gradually activate adipocyte metabolism and differentiation (Esteve Ràfols, 2014).

Also, adipogenesis may be inhibited by enhancing the degree of phosphorylation of AMPK-α, decreasing triglyceride content and suppressing C/EBPβ, a transcription factor that is commonly attributed to cellular PR peregrination regulation (Ali Redha et al., 2021).

In this study, western blotting revealed that *M. peregrina* seeds extract significantly enhances AMPK-α expression which in turn inhibited adipogenesis and reduce triglyceride content in tested models, also MP seeds oil suppressed C/EBPβ, a transcription factor responsible for activation of adipocyte metabolism and differentiation. Our results were in agreement with many previous studies (Kim et al., 2020; Muni Swamy et al., 2020; Xie et al., 2018).

In conclusion, *M. peregrina* seeds oil extract had a potential anti-obesity activity that may be attributed to different mechanisms. These included decreasing body weight, improving lipid levels by decreasing total cholesterol, triglycerides and LDL-C, and increasing HDL-C. Also, *M. peregrina* seeds extract regulated adipogenesis-associated genes, such as downregulating the expression of (PPARγ, C/EBPα, and FAS) and improving and upregulating the expression and phosphorylation of AMPKα and ACCI. Despite that *M. peregrina* seeds extract has reported clear anti-obesity potential through animal and laboratory studies, the available evidence-based studies on human clinical trials are very limited. Therefore, further studies are needed that could focus on clinical trials investigating anti-obesity potential mechanisms of *M. peregrina* seeds extracted in humans.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

ORCID

Naglaa Elarabany https://orcid.org/0000-0001-7977-5332

REFERENCES

Abd El Baky, H. H., & El-Baroty, G. S. (2013). Characterization of Egyptian *Moringa peregrine* seed oil and its bioactivities. *International Journal of Management Sciences and Business Research*, *2*, 98–108.

Ali Redha, A., Perna, S., Riva, A., Petrangolini, G., Peroni, G., Nichetti, M., Iannello, G., Naso, M., Faliva, M. A., & Rondanelli, M. (2021). Novel insights on anti-obesity potential of the miracle tree, *Moringa oleifera*: A systematic review. *Journal of Functional Foods*, *84*, 104600.

Alkhudhayri, D. A., Osman, M. A., Alshammari, G. M., Al Maiman, S. A., & Yahya, M. A. (2021). *Moringa peregrina* leaf extracts produce anti-obesity, hypoglycemic, anti-hyperlipidemic, and hepatoprotective effects on high-fat diet fed rats. *Saudi Journal of Biological Sciences*, *28*, 3333–3342.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*, *21*, 17–25.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., International Natural Product Sciences Taskforce, & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, *20*, 200–216.

Axen, K. V., & Axen, K. (2006). Very low-carbohydrate versus isocaloric high-carbohydrate diet in dietary obese rats. *Obesity (Silver Spring, Md.)*, *14*, 1344–1352.

Bais, S., Singh, G. S., & Sharma, R. (2014). Antiobesity and hypolipidemic activity of *Moringa oleifera* leaves against high fat diet-induced obesity in rats. *Advances in Biology*, *2014*, 2014.

Barbagallo, I., Vanella, L., Distefano, A., Nicolosi, D., Maravigna, A., Lazzarino, G., Di Rosa, M., Tibullo, D., Acquaviva, R., & Li Volti, G. (2016). *Moringa oleifera* Lam. improves lipid metabolism during adipogenic differentiation of human stem cells. *European Review for Medical and Pharmacological Sciences*, *20*, 5223–5232.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Cequier-Sánchez, E., Rodriguez, C., Ravelo, A. G., & Zarate, R. (2008). Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures. *Journal of Agricultural and Food Chemistry*, *56*, 4297–4303.

Chung, J., & Scanu, A. M. (1974). Continuous pH-stat titration method for the assay of lipoprotein lipase activity in vitro. *Analytical Biochemistry*, *62*, 134–148.

Dongmeza, E. B., Siddhuraju, P., Francis, G., & Becker, K. (2006). Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* [L.]). *Aquaculture*, *261*, 407–422.

Ebrahimi, R., Toolabi, K., Jannat Ali Pour, N., Mohassel Azadi, S., Bahiraee, A., Zamani-Garmsiri, F., & Emamgholipour, S. (2020). Adipose tissue gene expression of long non-coding RNAs; MALAT1, TUG1 in obesity: is it associated with metabolic profile and lipid homeostasis-related genes expression? *Diabetology & Metabolic Syndrome*, *12*, 36.

Fan, Q., Xu, F., Liang, B., & Zou, X. (2021). The anti-obesity effect of traditional Chinese medicine on lipid metabolism. *Frontiers in Pharmacology*, *12*, 696603.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*, 499–502.

Islam, Z., Islam, S. M. R., Hossen, F., Mahtab-Ul-Islam, K., Hasan, M. R., & Karim, R. (2021). *Moringa oleifera* is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*, *2021*, 6627265.

Khera, R., Murad, M. H., Chandar, A. K., Dulai, P. S., Wang, Z., Prokop, L. J., Loomba, R., Camilleri, M., & Singh, S. (2016). Association of pharmacological treatments for obesity with weight loss and adverse events: A systematic review and meta-analysis. *JAMA*, *315*, 2424–2434.

Kilany, O. E., Abdelrazek, H. M., Aldayel, T. S., Abdo, S., & Mahmoud, M. M. (2020). Anti-obesity potential of *Moringa oleifera* seed extract and lycopene on high fat diet induced obesity in male Sprauge Dawely rats. *Saudi Journal of Biological Sciences*, *27*, 2733–2746.

Kim, D. S., Choi, M. H., & Shin, H. J. J. (2020). Extracts of *Moringa oleifera* leaves from different cultivation regions show both antioxidant and antiobesity activities. *Journal of Food Biochemistry*, *44*, e13282.

Kim, J. H., Kim, H. J., Park, H. W., Youn, S. H., Choi, D. Y., & Shin, C. S. (2007). Development of inhibitors against lipase and alpha-glucosidase from derivatives of monascus pigment. *FEMS Microbiology Letters*, *276*, 93–98.

Kinnunen, T. I., Bastola, K., & Neupane, S. (2021). Trends in the prevalence of overweight and obesity among women of reproductive age. *Journal of Nepal Health Research Council*, *19*, 252–258.

Kostrycki, I. M., Wildner, G., Donato, Y. H., Dos Santos, A. B., Beber, L. C. C., Frizzo, M. N., Ludwig, M. S., Keane, K. N., Cruzat, V., Rhoden, C. R., & Heck, T. G. (2019). Effects of high-fat diet on eHSP72 and extra-to-intracellular HSP70 levels in mice submitted to exercise under exposure to fine particulate matter. *Journal of Diabetes Research*, *2019*, 4858740.

Lopez-Teros, V., Ford, J. L., Green, M. H., Tang, G., Grusak, M. A., Quihui-Cota, L., Muzhingi, T., Paz-Cassini, M., & Astiazaran-Garcia, H. (2017). Use of a "super-child" approach to assess the vitamin a equivalence of moringa oleifera leaves, develop a compartmental model for vitamin A kinetics, and estimate vitamin A total body

stores in young Mexican children. *The Journal of Nutrition*, *147*, 2356–2363.

Metwally, F. M., Rashad, H. M., Ahmed, H. H., Mahmoud, A. A., Raouf, E. R. A., & Abdalla, A. M. (2017). Molecular mechanisms of the anti-obesity potential effect of *Moringa oleifera* in the experimental model. *Asian Pacific Journal of Tropical Biomedicine*, *7*, 214–221.

Müller, T. D., Blüher, M., Tschöp, M. H., & DiMarchi, R. D. (2021). Anti-obesity drug discovery: Advances and challenges. *Nature Reviews Drug Discovery*, *20*, 1–23.

Muni Swamy, G., Ramesh, G., Devi Prasad, R., & Meriga, B. (2020). Astragalin, (3-O-glucoside of kaempferol), isolated from *Moringa oleifera* leaves modulates leptin, adiponectin secretion and inhibits adipogenesis in 3T3-L1 adipocytes. *Archives of Physiology and Biochemistry*, *126*, 1–7.

Nahar, S., Faisal, F. M., Iqbal, J., Rahman, M. M., & Yusuf, M. A. (2016). Antiobesity activity of *Moringa oleifera* leaves against high fat diet-induced obesity in rats. *International Journal of Basic & Clinical Pharmacology*, *5*, 1263–1268.

Ndhlala, A. R., Mulaudzi, R., Ncube, B., Abdelgadir, H. A., du Plooy, C. P., & Van Staden, J. (2014). Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* Lam. cultivars. *Molecules (Basel, Switzerland)*, *19*, 10480–10494.

National Research Council. (1995). *Nutrient requirements of laboratory animals: 1995*.

Nyberg, S. T., Batty, G. D., Pentti, J., Virtanen, M., Alfredsson, L., Fransson, E. I., Goldberg, M., Heikkilä, K., Jokela, M., Knutsson, A., Koskenvuo, M., Lallukka, T., Leineweber, C., Lindbohm, J. V., Madsen, I. E. H., Magnusson Hanson, L. L., Nordin, M., Oksanen, T., Pietiläinen, O., … Kivimäki, M. (2018). Obesity and loss of disease-free years owing to major non-communicable diseases: A multicohort study. *The Lancet Public Health*, *3*, e490–e497.

Onakpoya, I. J., Heneghan, C. J., & Aronson, J. K. (2016). Post-marketing withdrawal of anti-obesity medicinal products because of adverse drug reactions: A systematic review. *BMC Medicine*, *14*, 191.

Öztürk, N., Tuncel, M., & Tuncel, N. (2007). Determination of phenolic acids by a modified HPLC: Its application to various plant materials. *Journal of Liquid Chromatography & Related Technologies*, *30*, 587–596.

Ràfols, E. M. (2014). Adipose tissue: Cell heterogeneity and functional diversity. *Endocrinología y Nutrición* (English Edition), *61*, 100–112.

Sethi,J. K., & Vidal-Puig, A. J. (2007). Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. *Journal of lipid research*, *48*, 1253–1262.

Tejas, G., Umang, J., Payal, B., Tusharbinu, D., & Pravin, T. (2012). A panoramic view on pharmacognostic, pharmacological, nutritional, therapeutic and prophylactic values of *Moringa oleifera* Lam. *International Research Journal of Pharmacy*, *3*, 1–7.

Vongsak, B., Sithisarn, P., & Gritsanapan, W. (2013). Simultaneous determination of crypto-chlorogenic acid, isoquercetin, and astragalin contents in *Moringa oleifera* leaf extracts by TLC-densitometric method. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 917609.

Xie, J., Wang, Y., Jiang, W. W., Luo, X. F., Dai, T. Y., Peng, L., Song, S., Li, L. F., Tao, L., Shi, C. Y., Hao, R. S., Xiao, R., Tian, Y., & Sheng, J. (2018). *Moringa oleifera* leaf petroleum ether extract inhibits lipogenesis by activating the AMPK signaling pathway. *Frontiers in Pharmacology*, *9*, 1447.

Xie, Z., Cheng, Y., Zhang, Q., Hao, H., Yin, Y., Zang, L., Wang, X., & Mu, Y. (2021). Anti-obesity effect and mechanism of mesenchymal stem cells influence on obese mice. *Open Life Sciences*, *16*, 653–666.

Xu, Y.-B., Chen, G.-L., & Guo, M.-Q. (2019). Antioxidant and anti-inflammatory activities of the crude extracts of moringa oleifera from Kenya and their correlations with flavonoids. *Antioxidants*, *8*, 296.

Zhou, Y.-J., Xu, N., Zhang, X.-C., Zhu, Y.-Y., Liu, S.-W., & Chang, Y.-N. (2021). Chrysin improves glucose and lipid metabolism disorders by regulating the AMPK/PI3K/AKT signaling pathway in insulin-resistant HepG2 cells and HFD/STZ-induced C57BL/6J mice. *Journal of Agricultural and Food Chemistry*, *69*, 5618–5627.

Zhu, M. Z., Wu, W., Jiao, L. L., Yang, P. F., & Guo, M. Q. (2015). Analysis of flavonoids in lotus (*Nelumbo nucifera*) leaves and their antioxidant activity using macroporous resin chromatography coupled with LC-MS/MS and antioxidant biochemical assays. *Molecules (Basel, Switzerland)*, *20*, 10553–10565.

How to cite this article: Elarabany, N., Hamad, A., & AlSobeai, S. M. (2022). Evaluating anti-obesity potential, active components, and antioxidant mechanisms of *Moringa peregrina* seeds extract on high-fat diet-induced obesity. *Journal of Food Biochemistry*, *46*(10), e14265. https://doi.org/10.1111/jfbc.14265