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Molecular mechanisms of the anti-obesity potential effect of *Moringa oleifera* in the experimental model

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ABSTRACT

Objective: To elucidate the molecular mechanisms of the potent anti-obesity effect of *Moringa oleifera* Lam. (*M. oleifera*) ethanolic extract and to clarify the link between these mechanisms and the associated metabolic and vascular risks in the experimental model of visceral obesity.

Methods: *M. oleifera* ethanolic extract was orally administered at 600 mg/kg body weight in obese female rats daily for 12 weeks. At the end of treatment, body weight was determined, and the atherogenic index, coronary artery index, glucose level, insulin resistance status, liver and kidney functions were assessed. Also, the mRNA of leptin, adiponectin and resistin in visceral adipose tissue was determined by quantitative real time-PCR.

Results: The results showed that *M. oleifera* extract down-regulated mRNA expression of leptin and resistin, while it up-regulated adiponectin gene expression in obese rats relative to untreated obese control counterparts. This amelioration of genes expression was paralleled by a reduction in body weight and improvement of the atherogenic index and coronary artery index, as well as glucose level and insulin resistance value without adverse effects on liver or kidney functions, versus the untreated obese control ones.

Conclusions: It is reasonable to assume that the anti-obesity, anti-atherogenic and anti-diabetic properties of *M. oleifera* are mechanistically achieved via working directly on the adipokines of the visceral adipose tissue. Therefore, *M. oleifera* may be a good therapeutic candidate for the symptoms of metabolic syndrome.

1. Introduction

Obesity is one of major epidemic problems threatening health worldwide. Depending on fast foods of high cholesterol content, not only leads to atherogenic dyslipidemia but implicates in the pathogenesis of the rest of the metabolic syndrome components such as abdominal obesity, insulin resistance, and hypertension over time as well [1]. The visceral adipose tissue is an active endocrine organ that secretes adipokines like leptin, adiponectin and resistin which play the main role in energy expenditure, food intake, insulin secretion and sensitivity, metabolism, inflammation and endothelial function *etc*. [2]. Adipokines function through their effect on insulin-sensitive tissues such as

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brain, liver, muscle, endothelium, heart and pancreas. Visceral adiposity dysfunction causes changes in adipokines releasing levels that derive disturbances in the body metabolism, insulin sensitivity and cardiovascular abnormalities [2]. Thus, adipokines were considered potential targets for the therapeutic management of obesity and cardiovascular disease. The major discipline of the treatment of the cardiometabolic derangements like atherosclerosis is the management of total cholesterol (TC) and the attenuation of low density lipoprotein-cholesterol (LDL-C). Therefore, the use of cholesterol-lowering agents is overwhelming; the statins are the most famous therapy despite their adverse effects [3]. In contrast to those drugs, alternative therapy based on natural products and plant extracts has been used worldwide due to their minimal adverse effects and their efficiency in the treatment of hyperlipidemia [4]. Many of these extracts have been investigated as anti-obesity drugs and many of them show high therapeutic potency [5,6].

Moringa oleifera Lam. (*M. oleifera*) is a member of family Moringaceae, which is widely cultivated in tropical and subtropical areas. It is commonly known as miracle tree, since various parts of the plant including roots, leaves, and seeds possess various medicinal as well as nutritional values. Therefore, various preparations of *M. oleifera* exhibited antibiotic, hypotensive, anti-ulcer, anti-inflammatory and anti-cancer properties [7]. The edible leaves of *M. oleifera* tree have been known as an anti-diabetic food for centuries [8]. The aqueous extract of *M. oleifera* leaves demonstrated potent antioxidant and anti-diabetic activity [9]. Furthermore, the methanolic extract of *M. oleifera* leaves improved dyslipidemia and body weight gain in experimentally induced obesity in rats [6]. Recently, ethanolic extract of *M. oleifera* leaves has been shown to have hypocholesterolemic and antioxidant activities in obese rats [5].

In light of our previous work [5], it showed evidence for the anti-obesity activity of *M. oleifera* in improving body weight loss and amelioration of hyperleptinemia, hypoadiponectinemia and hyperresistinemia in obese rats. The present study was conducted to explore the underlying molecular mechanisms in favor of the anti-obesity potential of *M. oleifera* ethanolic extract in the experimental model of visceral obesity. This was achieved through studying the extract effects on gene expression levels of leptin, adiponectin and resistin in visceral adipose tissue and linking the molecular mechanisms with the associated metabolic and vascular risks.

2. Materials and methods

2.1. Plant material

M. oleifera (Family: Moringaceae) was provided by the Department of Horticulture Crops Technology, National Research Centre, Giza, Egypt. Meanwhile, identification of *M. oleifera* was accomplished at Botany Department, Faculty of Science, Cairo University, Giza, Egypt. A voucher herbarium specimen (MO-2014-13) was processed and stored for long-term maintenance at the herbarium museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

2.1.1. Preparation of *M. oleifera* extract

The aerial parts of *M. oleifera* weighing 2 kg were first air-dried and then ground into powder. The *M. oleifera* powder was treated with 95% ethanol (3 × 4 L) to be extracted via cold percolation. Then, the ethanol extract was concentrated under reduced pressure to yield 250 g of a brown residue that was stored in a refrigerator until used in the treatment of experimental animals.

2.1.2. Phenolic and flavonoids contents of *M. oleifera* extract

The phenolic content was determined by Folin–Ciocalteu colorimetric assay [10]. An aliquot (100 μL) of plant extract, 7 mL of deionized water and 0.5 mL of Folin–Ciocalteu reagent were mixed and incubated for 3 min at room temperature. Then, 1.5 mL of sodium carbonate (20% w/v) was added and the volume was completed to 10 mL by deionized water and incubated for 2 h at room temperature under dark condition. Gallic acid (1 mg/mL) was used as standard, and total phenolic content was calculated from the regression equation of the standard plot ($y = 116.52x - 0.067$, $r^2 = 0.9967$) and expressed in g gallic acid equivalent/100 g extract. The absorbance was determined at 725 nm. The result showed that the polyphenolic content of *M. oleifera* extract was 6.98 g gallic acid equivalent/100 g extract.

The aluminum chloride colorimetric method was used to determine flavonoids content [11]. A volume of 1 mL of sample plant extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 mol/L potassium acetate and 5.6 mL of distilled water and incubated at room temperature for 30 min. Rutin was used as standard (1 mg/mL). Flavonoids content was calculated from the regression equation of the standard plot ($y = 681.26x + 64.155$, $r^2 = 0.9897$) and expressed as rutin equivalent (g/100 g of extracted compound). The absorbance was measured at 420 nm. The result illustrated that the flavonoids content of *M. oleifera* extract was 2.85 g rutin equivalent/100 g extract.

2.2. Animals

Thirty-two adult female Wistar rats at 90 days of age [(130 ± 10) g] were obtained from a breeding stock maintained in the Animal House of the National Research Centre, Giza, Egypt. Animals were maintained in polypropylene cages under environmentally controlled conditions with a temperature of (24 ± 1) °C, a 12 h light/12 h dark cycle and a relative humidity of 60% ± 5%. They were fed with standard rat diet and water was provided *ad libitum*. Rats were allowed two weeks to acclimatize to animal room conditions before the commencement of the experiment. The experimental protocol was approved by Institutional Ethics Committee of the National Research Centre, Giza, Egypt and experiments were performed as per guidelines of National Research Centre Ethical Committee for Medical Research "Animal Experimentation Sector".

2.3. Experimental setting

After the acclimatization period, eight rats were chosen as the normal control (NC) group. They received water *ad libitum* and standard laboratory rodent diet (26.5% protein, 3.8% fat, 40% carbohydrate and 4.5% crude fiber) throughout the experimental period. The rest of the rats (twenty-four) received water *ad libitum* and high-cholesterol diet (HCD) (19.93% protein, 15.00% cholesterol, 57.50% carbohydrate and 2.81% dietary fiber) (Sigma Chemical Co., USA) for 12 consecutive weeks,

which was the same as our previous work [5]. The dietary constituents were homogenized in distilled water at 60 °C and the resulting homogenate was used to prepare the pellets. Diets were introduced fresh each day as dry pellets.

All rats were allocated into 4 groups (8 rats each) to undergo treatments as follows for 12 weeks: Group 1 (NC): normal control group administered with vehicle (distilled water) only; Group 2: untreated obese group orally administered with vehicle (distilled water) only (Ob); Group 3: obese group orally administered with ethanolic extract of *M. oleifera* (600 mg/kg body weight) [12] (Ob + *M. oleifera*) and Group 4: obese group orally administered with cholesterol-lowering drug (simvastatin), purchased from MSD B.V. Co., UAE (5 mg/kg body weight) [8] (Ob + Sim).

2.4. Sample collection

At the end of the experimental period, the final body weight of rats was recorded. Rats were euthanized after withholding food for 14 h, and the blood samples were withdrawn under diethyl ether anesthesia from the retro-orbital plexus in clean dry centrifuge tubes and allowed to clot to separate out the sera. Serum samples were separated by centrifugation at 4 000 r/min for 10 min at 4 °C. Aliquots of serum were frozen and stored at −20 °C for further determination of biochemical markers. Visceral adipose tissues were excised and weighed, then stored immediately in RNA later and frozen at −80 °C until used for gene expression analysis using quantitative real-time PCR (qRT-PCR).

2.5. Assessment of atherogenic index (AI) and cardiac risk index (CRI)

Serum TC and high density lipoprotein-cholesterol (HDL-C) levels were assayed by colorimetric method using Reactivos GPL kits (Barcelona, Espana) according to Meiattini *et al.* [13] and Naito and David [14] methods, respectively. Serum LDL-C level was assayed by colorimetric method using Centronic GmbH (Wartenberg, Germany) following the method of Wieland and Seidel [15]. These parameters were determined in order to calculate the AI and CRI as well, according to the following formulas LDL-C (mg/dL)/HDL-C (mg/dL) [16] and TC (mg/dL)/HDL-C (mg/dL) [17], respectively.

2.6. Assessment of insulin resistance

Serum glucose concentrations were estimated colorimetrically according to manufacturer's instructions, using Reactivos GPL kits (Barcelona, Espana). Serum insulin was assayed by ELISA using a kit purchased from Glory Science Co., Ltd., USA according to manufacturer's instructions. The homeostasis model assessment of insulin resistance (HOMA-IR) [18] was calculated as the product of the fasting serum glucose (mg/dL) and fasting insulin levels (μIU/mL) divided by a constant.

2.7. Assessment of liver and kidney functions

Liver functions [serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities] as well as kidney functions [serum levels of creatinine and blood urea nitrogen (BUN)] were determined according to the manufacturer's instructions. The previous kits were purchased from Spectrum Diagnostics, Egyptian Company for Biotechnology, Cairo, Egypt.

2.8. RNA extraction and cDNA synthesis

Visceral adipose tissues (100 mg each) were collected from 8 rats per group and stored for a maximum of 4 days in the RNAlater (RNA stabilizer reagent) solution (Qiagen, Germany). About 30 mg of stored samples were ground and homogenized using a mortar and pestle in liquid nitrogen. Total RNA isolation from tissues was performed using Thermo Scientific GeneJET RNA Purification Kit. RNA was quantified spectrophotometrically using ND-1000 Spectrophotometer (NanoDrop). cDNA was obtained from 1 μg sample of tissue-derived RNA (high capacity cDNA) with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) and the incubation was performed on the gradient thermal cycler (Bio-Rad).

2.9. qRT-PCR

qRT-PCR was carried out in Stratagene Mx3000P, Real-Time PCR System, Agilent Technologies. The qRT-PCR was performed in duplicate for each sample using Maxima SYBR Green qPCR Master Mix, Thermo Scientific. The reaction mixture was performed in a total volume of 20 μL containing 4 μL of cDNA (100 ng/μL), 300 nmol/L of each primer set for each gene, and 10 μL of SYBR Green Master Mix and completed to 20 μL with nuclease-free water. Each gene expression was normalized with the housekeeping gene β-actin. The primer sequences for different genes (synthesized by Biosearch Technologies, USA) are listed in Table 1. The thermal cycler program was as follows: 95 °C for 5 min and 40 cycles of 94 °C for 15 s, annealing for 60 s according to melting temperature suitable for each primer set, extension at 72 °C for 10 s. The $2^{-\Delta\Delta CT}$ formula, the method of relative quantification of mRNA was used to determine the fold difference in gene expression.

Table 1
Primer sequences of the studied genes.

Gene	Primer sequence (5′–3′)	Annealing temperature (°C)	GenBank accession number	References
Leptin	Forward CTCAGCATTCAGGGCTAAGG Reverse AAGCCTCGCTCTACTCCACA	62	NM_013076	[19]
Adiponectin	Forward AATCCTGCCCAGTCATGAAG Reverse CATCTCCTGGGTCACCCTTA	65	NM_144744	[20]
Resistin	Forward AGTTGTGCCCTGCTGAGCTCTCTGCC Reverse CCCATTGTGTATTTCCAGACCCTC	62	TC_119941	[21]
β-Actin	Forward GTGGGGCGCCCCAGGCACCA Reverse CTCCTTAATGTCACGCACGATTTC	60	NM_031144	[19]

2.10. Statistical analysis

Analyses were performed by SPSS version 16 software for Windows (SPSS, Chicago, IL). Data were expressed as mean ± SEM. Differences between groups were tested for statistical significance using One-way ANOVA, followed by Tukey's test. A $P < 0.05$ was considered significant for all data analyses.

3. Results

3.1. Effect of M. oleifera on body weight, AI and CRI

The initial body weight was similar among all the experimental groups. The final body weight of the obese group increased ($P < 0.05$) significantly compared to the normal control group. On the contrary, oral administration of the ethanolic extract of *M. oleifera* (600 mg/kg body weight) or simvastatin (5 mg/kg body weight) for 12 consecutive weeks decreased body weight of obese rat ($P < 0.05$) significantly, relative to the untreated obese counterparts (Figure 1A).

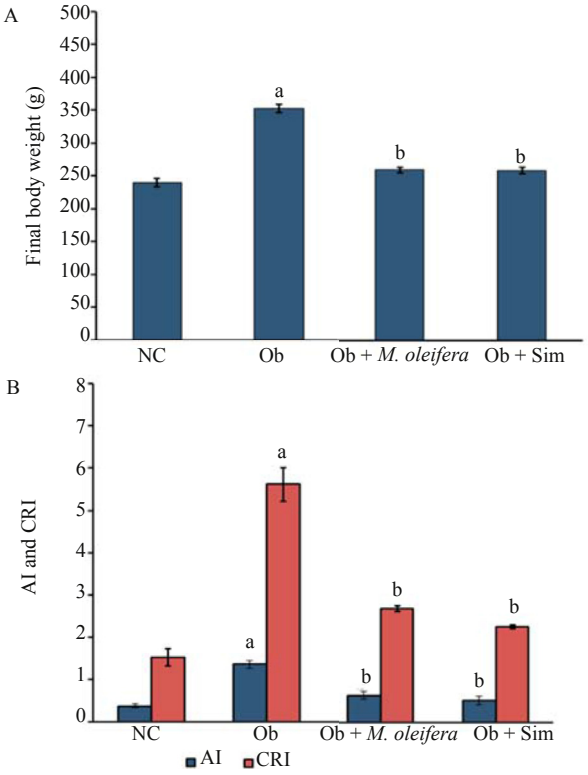

Figure 1. Effect of ethanolic extract of *M. oleifera* on final body weight (A), and AI and CRI (B).
Data are represented as mean ± SEM ($n = 8$). [a]: Significant change at $P < 0.05$ in comparison with NC group; [b]: Significant change at $P < 0.05$ in comparison with Ob group.

The AI and CRI were significantly ($P < 0.05$) higher in obese rats than in the normal control ones. Oral administration of *M. oleifera* or simvastatin significantly ($P < 0.05$) lowered both values versus those recorded in the untreated obese counterparts (Figure 1B).

3.2. Effect of M. oleifera on serum glucose level and insulin resistance value

Female obese rats showed elevated serum glucose, insulin levels and HOMA-IR value. Thus, the obese group was developing insulin resistance, compared to the normal control group ($P < 0.05$) (Figure 2). *M. oleifera* or simvastatin treatment in the obese rats normalized blood glucose and insulin levels and ameliorated insulin resistance value as shown by the reduced HOMA-IR score ($P < 0.05$) (Figure 2).

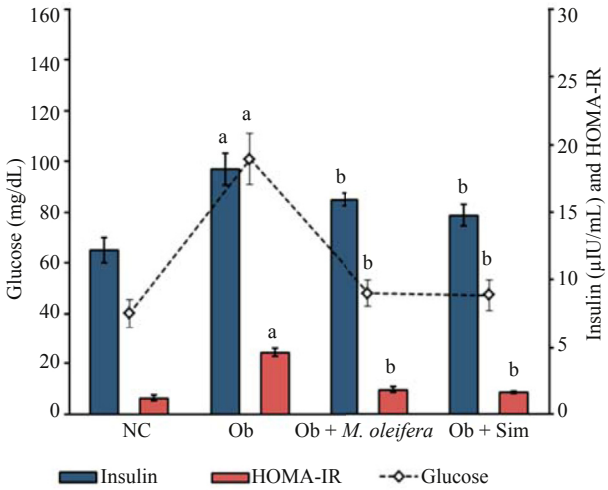

Figure 2. Effect of ethanolic extract of *M. oleifera* on serum glucose, and insulin and HOMA-IR of female obese rats.
Data are represented as mean ± SEM ($n = 8$). [a]: Significant change at $P < 0.05$ in comparison with NC group; [b]: Significant change at $P < 0.05$ in comparison with Ob group.

3.3. Effect of M. oleifera on liver and kidney functions

Results of liver functions illustrated that the obese group showed significant elevation in serum transaminases (ALT and AST) activity relative to the normal control group ($P < 0.05$). The ethanolic extract of *M. oleifera* ameliorated the activity of the previous hepatic biomarkers ($P < 0.05$) significantly, compared to the untreated obese group. Also, simvastatin exhibited significant improvement in serum AST activity compared to the untreated obese group ($P < 0.05$). However, the activity of ALT in serum was significantly ($P < 0.05$) increased upon simvastatin treatment compared to the untreated obese group ($P < 0.05$) (Table 2).

Obese rats showed insignificant ($P > 0.05$) changes in serum creatinine and BUN levels compared to normal control counterparts. Also, *M. oleifera* or simvastatin treatment exhibited insignificant changes ($P > 0.05$) in serum creatinine and BUN levels compared to the untreated obese group (Table 2).

3.4. Effect of M. oleifera extract on expression of leptin, adiponectin and resistin genes in adipose tissue

Comparative gene expression analysis by RT-PCR showed that leptin and resistin gene expression in obese group was up-regulated to (1.79 ± 0.09) fold and (1.20 ± 0.07) fold compared to the normal control group, respectively ($P < 0.05$) (Figure 3A, B). While, adiponectin gene expression was down-regulated in the obese rats reaching (0.28 ± 0.01) fold compared to the normal control counterparts ($P < 0.05$) (Figure 3C).

M. oleifera extract or simvastatin treatment in obese rats showed significant down-regulation of the leptin gene expression reaching (0.41 ± 0.02) fold and (0.44 ± 0.02) fold compared to the untreated obese group, respectively ($P < 0.05$) (Figure 3A). Also, both treatments exhibited down-regulation

Table 2
Effect of ethanolic extract of *M. oleifera* on liver (ALT and AST) and kidney functions (creatinine and BUN) of female obese rats.

Treatment	ALT (IU/mL)	AST (IU/mL)	Creatinine (mg/dL)	BUN (mg/dL)
NC	38.26 ± 3.98	66.11 ± 6.30	0.80 ± 0.38	15.54 ± 2.88
Ob	74.81 ± 5.02[a]	131.15 ± 11.51[a]	0.90 ± 0.26	17.76 ± 1.51
Ob + *M. oleifera* (600 mg/kg body weight)	44.90 ± 3.59[b]	72.64 ± 8.79[b]	0.88 ± 0.30	15.81 ± 1.32
Ob + Sim (5 mg/kg body weight)	76.30 ± 4.77[a,b]	70.49 ± 10.50[b]	0.91 ± 0.49	16.09 ± 1.77

Data are represented as mean ± SEM (n = 8).
[a]: Significant change at $P < 0.05$ in comparison with NC group; [b]: Significant change at $P < 0.05$ in comparison with Ob group.

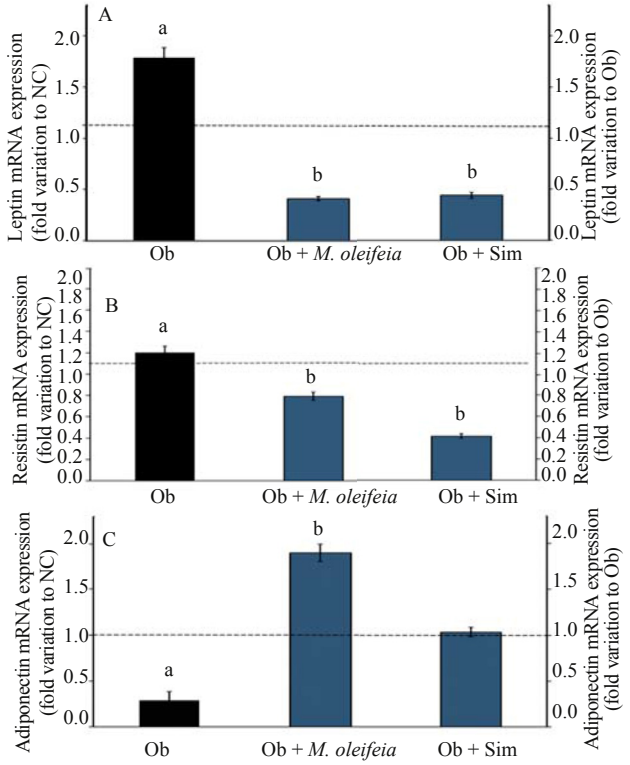

Figure 3. Effect of ethanolic *M. oleifera* extract on leptin (A), resistin (B) and adiponectin (C) mRNA expression in adipose tissue of female obese rats.
The fold variation of mRNA in Ob group is compared with the NC condition (dashed line), while the fold variation of mRNA in Ob + *M. oleifera* and Ob + Sim is compared with the Ob condition. Data are represented as mean ± SEM (n = 8). A: Significant change at $P < 0.05$ in comparison with NC group; B: Significant change at $P < 0.05$ in comparison with Ob group.

($P < 0.05$) of the resistin gene expression reaching (0.84 ± 0.05) fold and (0.42 ± 0.02) fold compared to the untreated obese group (Figure 3B). On the other side, *M. oleifera* extract caused a significant ($P < 0.05$) up-regulation of adiponectin mRNA expression in the obese rats; the expression difference was (1.91 ± 0.07) fold compared to the untreated obese counterparts (Figure 3C). Whereas, simvastatin administration showed insignificant ($P > 0.05$) change in the adiponectin gene expression relative to the untreated obese group.

4. Discussion

Visceral adiposity is regarded as the direct link between obesity and several metabolic diseases, including risk of incidence of type 2 diabetes, atherosclerosis and hypertension, which are totally clustered under the clinical signs of the metabolic syndrome. Our previous study [5] confirmed the effectiveness of ethanolic extract of *M. oleifera* in improving obesity and dyslipidemia occurred in female obese rats after long-term treatment with HCD. Also, *M. oleifera* showed beneficial impact in ameliorating hyperleptinemia, hyperresistinemia, and hypoadiponectinemia in the serum of obese rats [5]. Therefore, the present study was constructed to clarify the underlying molecular mechanisms of the anti-obesity effect of ethanolic extract of *M. oleifera* in the same experimental model of visceral obesity and justify the link between these mechanisms and the metabolic as well as vascular risks associated with obesity.

The present data showed a development of frank obesity in female rats fed on a HCD relative to the normal control counterparts at the end of the experimental period, confirming the previous studies that associated the long-term dependence of rats on a HCD and induction of obesity [4]. Further, the obese group developed insulin resistance with obvious hyperglycemia and higher HOMA-IR score. Moreover, it exhibited high AI and CRI relative to normal control ones. In addition, obesity caused hepatic damage manifested by the elevation of serum transaminases activity [22]. Serum creatinine and BUN values of obese rats were not significantly different from those of normal control counterparts, illustrating that HCD is not nephrotoxic to rats. Thus, obese rats displayed the main features of the metabolic syndrome and early features of the type 2 diabetes [1].

The current study revealed that administration of ethanolic extract of *M. oleifera* in obese females attenuated body weight gain and both AI and CRI. Thus, it ameliorated obesity and atherogenic dyslipidemia. This was in accordance with Bais *et al.* [6], who recorded a reduction of AI upon administration of methanolic extract of *M. oleifera* leaves. This would make *M. oleifera* efficient as simvastatin, the well-known cholesterol-lowering drug. Simvastatin inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase, the master enzyme of the cholesterol synthesis pathway [23]. Hence, it reduces atherogenicity and cardiovascular mortality, via improving lipid profile particularly TC, LDL-C and promotion of HDL-C synthesis [5].

Furthermore, ethanolic extract of *M. oleifera* and simvastatin improved insulin resistance and symptoms of type 2 diabetes mellitus observed in female obese rats. This was consistent with the recent study which recorded the role of *M. oleifera* leaves in modulating impaired insulin sensitivity and dyslipidemia observed in C57BL/6L mice fed high-fat diet [24].

Similarly, *M. oleifera* displayed hepatoprotective effect on the liver of obese female rats, represented by a marked reduction in ALT and AST enzymes activity in serum. This result is in parallel with the work of Fakurazi *et al.* [25], who reported the hepatoprotective activity of *M. oleifera* against hepatotoxicity induced by acetaminophen in rats. On the other hand, simvastatin as a reference drug caused to some extent a hepatic damage, which was attributed to impairment of ALT activity as seen in the present study and mentioned by Yang

et al. [3]. Furthermore, *M. oleifera* extract and simvastatin seem to be safe for kidney and away from nephrotoxicity, which is indicated by the results of creatinine and BUN, which are very close to normal control values.

The direct possible mechanism for the explanation of the therapeutic role of *M. oleifera* in improving the recorded metabolic derangements is the nature of *M. oleifera* as an antioxidant. *M. oleifera* represents a source of vitamins, minerals, amino acids, carotenoids, alkaloids, and flavonoids and contains phenolic compounds, such as zeatin, quercetin, isoquercetin, kaempferol, apigenin and rutin. These active ingredients accounted for the glucose-lowering effect of *M. oleifera* [9], hypolipidemic activity, reduction of atherogenesis and cardiovascular complications in a pre-clinical report as well [26].

Parallel to the described mechanism, the current data present another possible and more precise one, which is related directly to the expression levels of visceral adipose tissue adipokines and their burden on provoking versus improving the metabolic syndrome. Our hypothesis is supported by the results obtained from our previous work [5] in the same set of animals. Ahmed *et al.* [5] recorded a significant increase in body mass index and abdominal circumference of the female obese rats compared to the normal control counterparts, which was in accordance with the observed increase in final body weight of the same rats in the current report. These findings indicated the accumulation of abdominal fats and incidence of visceral obesity.

The visceral adipose tissue was chosen to be studied at the gene expression level due to its anatomical position and pattern of secretion. The deep visceral or mesenteric adipose tissue in rats resembles the omental tissue in human as they act as active endocrine glands. Their secretions (adipokines) above and beyond free fatty acids are collected by the portal vein, reaching the liver and contribute to the increased chronic inflammation, hepatic injury and impairment of liver enzymes, insulin resistance and cardiovascular risk associated with obesity [2]. Thus, the gene expression analysis of the leptin, resistin and adiponectin would give a causal correlation between visceral obesity and symptoms of metabolic syndrome.

Ahmed *et al.* [5] demonstrated the incidence of hyperleptinemia, hypoadiponectinemia and hyperresistenemia in female obese rats compared with the normal control counterparts. The present results confirmed the above-mentioned study and evidenced the molecular relationship between visceral obesity and cardiometabolic risk. In the visceral adipose tissue of obese rats, leptin and resistin gene expression was increased while adiponectin expression was decreased compared to the expression observed in the normal control rats. The treatment with *M. oleifera* extract down-regulated leptin and resistin mRNA expression and on the contrary, it up-regulated adiponectin mRNA expression. Similarly, simvastatin exhibited the same effect of *M. oleifera* extract, except it showed no effect towards the recovery of adiponectin gene expression.

Decreased leptin and resistin gene expression upon treatment with *M. oleifera* provided the main pathway in the improvement of adiposity. Previous studies suggested a two-way relationship between serum leptin level and leptin mRNA in adipocytes and adiposity level [27,28]. Luvizotto Rde *et al.* [28] cited that consumption of high-fat diets for a long duration caused increased expression of leptin and resistin which attributed to the increased adipose tissue mass. Meanwhile, a recent study showed that the increased serum leptin as a result of leptin mRNA and protein up-regulation promoted rats to exhibit high adiposity index upon feeding on high-sucrose diet [27]. Furthermore, up-regulation of adiponectin mRNA showed improvement in adiposity of the obese rat. Adiponectin mRNA down-regulation is a symptom of visceral adipose tissue accumulation in obese rats and mice and impairment of insulin sensitivity. Adiponectin expression is restored to normal levels after body fat mass reduction that evidenced through dual-energy X-ray absorptiometry [29].

In consideration of the thermogenic effect of *M. oleifera* on adipose tissues [24], the reduction of body weight, diminution in abdominal circumference as noted previously [5] and improvement of mRNA expression of the chosen adipokines in the obese group treated with *M. oleifera* would be explained. Our hypothesis is directly related to the attenuation of the mRNA expression of leptin as well as leptin resistance [5]. Previous reports showed that leptin-deficient *ob/ob* mouse showed increased food intake, accompanied by decreased metabolic rate, and a decreased core body temperature and increased weight gain in such a way that resembles the symptoms of leptin resistance [18,30]. Parallel results of Bais *et al.* [6] showed that administration of *M. oleifera* raised the body temperature and decreased body weight. Moreover, Waterman *et al.* [24] showed that isocyanates-rich *Moringa* and *Moringa* concentrate increased lipolysis and thermogenesis; therefore, fat accumulation and body mass were lowered.

The obvious therapeutic role of ethanolic extract of *M. oleifera* in the treatment of atherogenic dyslipidemia, insulin resistance and early sign of type 2 diabetes in obese rats is not far apart the improvement of leptin and resistin mRNA expression. Leptin resistance promotes fat deposition in liver, heart, and pancreas. Visceral and ectopic fat release free fatty acids into the blood and due to lipolysis, visceral adipose secretes more adipokines, such as tumor necrosis factor-α, interleukin-6, and C-reactive protein which promote inflammation, oxidative damage, and atherosclerosis [31]. Also, overconsumption of fats enhances hypothalamic resistance to anorexigenic hormones, leptin and insulin resulting in progressive fat accumulation, particularly at the visceral area and impaired insulin sensitivity. Therefore, leptin resistance, hyperresistinemia, and hypoadiponectinemia are implicated in the development of microvascular complications among obese and prediabetic subjects [32].

Moreover, resistin has been shown to function as a key factor in the development of insulin resistance and diabetes in rodents [33]. Recent reports demonstrated that mice subsequently fed a high-fat diet experienced higher plasma resistin levels, which is associated positively with changes in the intra-abdominal and subcutaneous fat pads [34]. Studies on rodents suggested that resistin is implicated in pathogenesis of insulin resistance by induction of 5′ AMP-activated protein kinase-dependent and AMP-activated protein kinase-dependent-independent suppressor of cytokine signaling 3 signaling pathways, leading to increase of gluconeogenesis through glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, elevation of fatty acid esterification of triglycerides, and initiation of fatty acids biosynthesis via acetyl-CoA carboxylase-1 as well [35]. Thus, interestingly *M. oleifera* treatment decreased glucose-6-phosphatase and phosphoenolpyruvate carboxykinase gene expression in C57BL/6 mice fed high-fat diet, which reduced gluconeogenesis and improved insulin sensitivity, like metformin's mode of action in the treatment of insulin resistance [36].

Additionally, *M. oleifera* might act as an insulin sensitizer as it behaves in a similar way to anti-resistin antibody, which enhances insulin-mediated glucose uptake in adipocytes, and to

rosiglitazone (the insulin-sensitizing peroxisome proliferator-activated receptor gamma agonist) which causes reduction in resistin gene expression and its secretion from the adipose tissue is decreased in *db*/*db* obese mice [37].

Additional important effect of up-regulation of adiponectin gene expression after treatment with *M. oleifera* was the reduction of the metabolic abnormalities occurred in obese rats. Adiponectin is an antiatherogenic agent that attenuates cardiovascular risk which may be attributed to its anti-inflammatory properties. It inhibits endothelial adhesion and dysfunction, because it suppresses the expression of "LDL scavenger receptors" on macrophages, thus lowering LDL uptake and plaque formation [38]. Contrary to leptin and resistin, adiponectin improves insulin sensitivity, and its concentration in serum is inversely proportional to visceral adiposity level. Obese patients with inherited hypoadiponectinemia developed a pattern of leptin resistance followed by insulin resistance, dyslipidemia and cardiovascular disease [39,40].

In conclusion, gene expression analysis provided a mechanistic therapeutic role of ethanolic extract of *M. oleifera* in the management of obesity and reduction of cardiometabolic abnormalities, resulted from HCD in female rats. *M. oleifera* extract worked directly on the visceral fat mass and ameliorated the impairment of mRNA expression of leptin, resistin and adiponectin genes. Thus, it showed improvement in body weight, atherogenic dyslipidemia and insulin resistance. Therefore, *M. oleifera* could be introduced as potent phytotherapy that could be comparable to simvastatin in the improvement of hypercholesterolemia, atherosclerosis and type 2 diabetes but without adverse effects and hepatic toxicity complication.

Conflict of interest statement

We declare that we have no conflict of interest.

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