Research Article

Moringa oleifera offers a Multi-Mechanistic Approach for Management of Obesity in Rats

Hanaa H. Ahmed[1]*, Fateheya M. Metwally[2], Hend Rashad[2] Asmaa M. Zaazaa[3], Shahira M. Ezzat[4], Maha M. Salama[4]
[1]Department of Hormones, National Research Centre, Cairo, Egypt.
[2]Environmental and Occupational Medicine, National Research Centre, Cairo, Egypt
[3]Department of Zoology, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
[4]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
***Corresponding author's E-mail:** hanaaomr@yahoo.com

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ABSTRACT

Obesity is a condition in which excess body fat is accumulated to an extent that health may be negatively affected. Obesity is associated with a number of chronic health problems such as diabetes, heart disease, hypertension and cancer. The current study was constructed to evaluate the efficacy of alcoholic extract of *Moringa oleifera* in management of obesity induced by high cholesterol diet in rats. Adult female albino rats were classified into four groups. The first group was kept on standard rodent chow for 30 weeks (lean control). The other three groups received high cholesterol diet for 30 weeks. These animals were assigned as obese control group, *Moringa oleifera* treated group and Simvastatin treated group. The results revealed significant increase in thoracic (TC) and abdominal (AC) circumferences as well as body mass index (BMI) in obese group. Moreover, dyslipidemia, and hyperleptinemia have been demonstrated in obese group. Furthermore, serum malondialdehyde (MDA) and nitric oxide (NO) levels were significantly increased in obese group versus the lean control group. In addition, obese group revealed significant decrease in serum adiponectin level in concomitant with significant increase in resistin level as compared to lean control group. On the other side, treatment with *Moringa oleifera* alcoholic extract or simvastatin could reduce food intake and BMI as well as ameliorate the dyslipidemia, in obese groups. Serum leptin level showed significant decrease in obese group due to treatment with *Moringa oleifera* alcoholic extract or Simvastatin. As well significant inhibition of serum MDA and NO levels was detected as a consequence of treatment with either *Moringa oleifera* extract or Simvastatin. Additionally, the treatment of obese group with *Moringa oleifera* extract or Simvastatin resulted in significant decrease in serum resistin level in concomitant with significant increase in serum adiponectin level as compared to obese control group. In conclusion, the data of the current study provides experimental evidence for the anti-obesity effect of *Moringa oleifera* ethanol extract. Thus, present findings reinforce the advice recommending consumption of *Moringa oleifera* to modulate obesity.

Keywords: Dyslipidemia, Malondialdehyde, *Moringa oleifera,* Nitric oxide, Obesity, Simvastatin.

INTRODUCTION

According to the World Health Organization (WHO) criteria, obesity is defined by a body mass index (BMI) of 30 kg/m2 or greater. A BMI above the healthy range of 18-25 kg/m2 is common in Western cultures and has been linked to both consumption of a Western diet (i.e. high saturated fat, high calorie content), and sedentary lifestyles.[1] Individual is considered obese when the amount of fat tissue is increased to such an extent that physical and mental health are affected and life expectancy reduced.[2]

The prevalence of overweight and obesity is increasing at an alarming rate in developed and developing countries throughout the world. The World Health Organization (WHO) predicts that by 2015, ~2.3 billion adults will be overweight and >700 million will be obese. Obesity rates in adolescents are also increasing with 200 million school-age children overweight globally.[3] Furthermore, the highly prevalent rate of obesity is not only in middle-aged adults, but also in children and young adults.[4]

Recent investigations suggested that the causes of obesity involve a complex interplay of genetic, environmental, psycho behavioral, endocrine, metabolic, cultural, and socioeconomic factors. Several genes and their protein products, such as leptin, may be particularly important in appetite and metabolic control, although the genetics of human obesity appear to involve multiple genes and metabolic pathways that require further elucidation. Obesity increases the risk of several leading causes of death and diseases in the world.[5] Severe obesity is frequently associated with significant comorbid medical conditions, including coronary artery disease, hypertension, type II diabetes mellitus, gallstones, nonalcoholic steatohepatitis, pulmonary hypertension, and sleep apnea and certain cancer types.[6,7]

For many obese individuals, diet and behavioral modification need to be supplemented by pharmacotherapy, or increasingly, bariatric surgery, because attempted adherence to a balanced diet and healthy lifestyle has not addressed this problem.[3] Therefore, increasing obesity levels will impose enormous health, financial and social burdens on worldwide society unless effective interventions are implemented.

Clinical guidance on the use of anti-obesity drugs stated that they should be an adjunct to first-line of treatments, that are, exercise and lifestyle modification.[8] There are

two different types of obesity-treatment drugs which are currently available on the market.[9] One of these is orlistat (Xenical), which reduces intestinal fat absorption through inhibition of pancreatic lipase.[10,11] The other is sibutramine (Reductil), which is an anorectic, or appetite suppressant.[12,13] Both drugs have side effects, including increased blood pressure, dry mouth, constipation, headache, and insomnia.[14,15] A number of anti-obesity drugs are currently undergoing clinical development, including centrally-acting drugs (e.g. radafaxine and oleoyl-estrone), drugs targeting peripheral episodic satiety signals (e.g. rimonabant and APD356), drugs blocking fat absorption (e.g. cetilistat and AOD9604), and human growth hormone fragments.[16,17]

At present, because of dissatisfaction with high costs and potentially hazardous side effects, the potential of natural products for treating obesity is under exploration, and this may be an excellent alternative strategy for developing future effective, safe anti-obesity drugs.[18,19] A variety of natural products, including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity.[20,21]

Moringa oleifera Lam. Syn. Moringa pterygosperma Gaerth (Moringaceae) is commonly used in traditional system of medicine as healing herb to treat diabetes. *Moringa oleifera* Lam is native to south Asia, but grows in tropical Africa and Latin America.[22,23] Different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human ailments. The leaves of *Moringa oleifera* are reported to be used as hypocholesterolemic and hypoglycemic remedy.[24,25]

The current study was constructed to evaluate the therapeutic potential and the possible mode of action of *Moringa oleifera* ethanolic extract in the treatment of obesity in adult female albino rats. This goal could be achieved through using anthropometric measurements and testing the hypothesis that the anthropometric index may predict obesity adverse effects on lipid profile and the effectiveness of the herbal therapy in the treatment of obesity in rats.

MATERIALS AND METHODS

Materials

Plant material

The aerial parts of *Moringa oleifera* Lam. (Family: Moringaceae) were obtained from the farm of the National Resaerch Center (Nubaria) during spring 2014. The plant was kindly identified at the Department of Botany, Faculty of Science, Cairo University, Giza, Egypt. A voucher specimen (MO-2014-13) is deposited at the museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Preparation of Moringa oleifera extract

The air-dried powdered aerial parts of *Moringa oleifera* (2 kg) were extracted by cold percolation with 95 % ethanol (3 x 4 L) till exhaustion. The ethanol extract was concentrated under reduced pressure to give 250 g of a brown residue. The residue was kept in a refrigerator till used in the biological study.

Chemicals and drugs

Cholesterol and sodium chloride were purchased from Sigma Chemical Co., USA. Simvastatin was purchased from MSD B.V Co., UAE.

Experimental set-up

This study was conducted in accordance with the principles and guidelines of the Ethical Committee for animal care and protection of the National Research Centre, Egypt.

Animals and experimental protocol

Thirty two adult female albino rats of Wistar strain weighing 130±10 g at 90 days of age were enrolled in the present study. The animals were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt in January 2014. The animals were housed 8 rats/cage in polypropylene cages in an environmentally controlled clean air room with a temperature of 24±1°C, a 12 h light/12 h dark cycle, a relative humidity of 60±5% and free access to tap water and food. Rats were allowed to adapt to these conditions for two weeks before beginning the experimental protocol.

After the acclimatization period, eight rats were given water *ad libitum* and fed a standard rodent chow with 26.5% protein, 3.8% fat, 40% carbohydrate, 4.5% crude fiber in 100 g of chow during 24 weeks of the experimental period and served as lean control group; G1 (Con group). The other twenty four rats were received water *ad libitum* and were fed a high-cholesterol diet (HCD) with 19.93% protein, 15% cholesterol, 57.50% carbohydrate, 2.81% dietary fiber in 100 g of chow [modified method of.[26] The dietary ingredients were homogenized in distilled water at 60°C and the homogenate was used to prepare the pellets. Diets were given fresh each day as dry pellets; therefore there was no spillage.[27] These rats served as obese groups which were further assigned into three groups; G2 (Ob group) in which the rats were fed a HCD diet for 12 weeks and left untreated for the other 12 weeks, G3 (Ob+*M. oleifera*) in which the rats were fed a HCD diet for 12 weeks, then they were orally administered with *Moringa oleifera* ethanolic extract in a dose of 600 mg kg^{-1} b.wt. according to Jain et al.[28] for 12 weeks and G4 (Ob+Sim) in which the rats were fed a HCD diet for 12 weeks, then they were orally administered with the anti-hypercholesterolemic drug Simvastatin at a daily dose of 5 mg kg^{-1} b.wt. According to Mbikay,[29] for 12 weeks.

Methods

Anthropometrical measurements

At the end of the experimental period, rats were fasted overnight (12-14 h) and the abdominal circumference (AC) (immediately anterior to the forefoot), thoracic circumference (TC) (immediately behind the foreleg), body length (nose-to-anus or nose-anus length) were measured in anaesthized rats. The body weight and body length were used to determine the body mass index.[27]

$$BMI = \frac{\text{Body weight (g)}}{\text{Length}^2\,(\text{cm}^2)}$$

Biochemical determinations

After taking the anthropometric parameters, the blood samples were withdrawn from the retro-orbital pleux in a clean dry centrifuge tubes and allowed to clot to obtain the sera. Serum samples were separated by centrifugation at 1800 xg for 10 min at 4°C. Aliquots of serum were frozen and stored at -20°C for further determinations of biochemical markers.

Serum total cholesterol (TC) and triglycerides (TG) and high density lipoprotein (HDL) levels were assayed by colorimetric method using Reactivos GPL kits (Barcelona, Espana) according to Meiattini,[30] Buccolo et al.[31] and Naito,[32] methods respectively. Serum low density lipoprotein (LDL) level was assayed by colorimetric method using Centronic (Gmbh) kit (Wartenberg, Germany) according to Wieland and Seidel[33] Serum malondialdehyde (MDA) level was determined by colorimetric method using Biodiagnostic kit (Egypt) according to the method described by Satoh[34] Serum nitric oxide (NO) level was determined by colorimetric method using Biodiagnostic kit (Egypt) according to the method described by Montgoery and Dymock.[35] Serum leptin, resistin and adiponectin were assayed by enzyme linked immunosorbent assay (ELIZA) technique using Gscience kits purchased from Glory Science Co., Ltd, USA, according to manufacturer's instruction.

Statistical analysis

In the present study, all results were expressed as mean ± S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 14.0 was used to compare significance between each two groups. Difference was considered significant when P <0.05. Percentage difference representing the percent of variation with respect to corresponding control group was also calculated.

$$\textbf{\% Difference} = \frac{\textbf{Treated value – Control value X 100}}{\textbf{Control value}}$$

RESULTS

The results of the current study revealed that there is significant increase in the thoracic circumference (TC), abdominal circumferences (AC) and BMI (P<0.05) of obese group with respect to the lean control group. On the other hand, there is significant decrease in TC, AC and BMI (P<0.05) of obese groups treated with the ethanolic extract of *Moringa oleifera* or Simvastatin versus the obese control group (Table 1). Noteworthy, there are insignificant changes (P>0.05) between obese group treated with ethanolic extract of *Moringa oleifera* and obese group treated with Simvastatin with respect to the anthropometric measurements in the current work (Table 1).

Table 1: Effect of treatment with ethanolic extract of *Moringa oleifera* on the anthropometric measurements of obese female rats. Data were represented as Mean ± S.E of 8 rats/group.

Groups	Parameters		
	TC (Cm)	AC (Cm)	BMI (g/Cm^2)
Lean control group	11.50 ± 0.27	14.25 ± 0.16	0.600 ± 0.015
Obese control group	15.00 ± 0.27[a] a(30.43%)	18.25 ± 0.25[a] a(28.07%)	0.880 ± 0.0146[a] a(46.71%)
Ob+*M. oleifera*	12.62 ± 0.26[b] b(-15.87%)	14.87 ± 0.23[b] b(-18.52%)	0.648 ± 0.010[b] b(-26.36%)
Ob+Sim	12.12 ± 0.23[b] b(-19.20%)	14.62 ± 0.26[b] b(-19.89%)	0.646 ± 0.013[b] b(-26.59%)

a: Significant change at P< 0.05 in comparison with the lean control group; b: Significant change at P< 0.05 in comparison with obese control group.

The results depicted in Table 2 showed the effect of treatment with ethanolic extract of *Moringa oleifera* on the lipid profile of obese female rats. The data revealed that there is significant increase in serum cholesterol, LDL and triglycerides levels (P<0.05) in obese group when compared with the lean control group. While, serum HDL level recorded significant decrease (P<0.05) in obese group as compared to the lean control group. In contrast, treatment of obese group with the ethanolic extract of *Moringa oliefera* or with Simvastatin elicited significant decrease in serum cholesterol, LDL and triglycerides levels (p<0.05) when compared with those in the obese control group. The opposite was observed regarding serum HDL level which showed significant increase (p<0.05) in group treated with *Moringa oleifera* ethanolic extract or with Simvastatin in comparison with that recorded in the obese control group (Table 2). Noteworthy, the obese group treated with *Moringa oleifera* ethanolic extract showed significant elevation in serum cholesterol, triglycerides and LDL levels (p<0.05) as compared to obese group treated with Simvastatin. While, serum HDL level showed insignificant change (P>0.05) in obese group treated with *Moringa oleifera* ethanolic extract in comparison with the obese group treated with Simvastatin (Table 2).

Table 2: Effect of treatment with ethanolic extract of *Moringa oleifera* on lipid profile of obese female rats. Data were represented as Mean ± S.E of 8 rats/group.

Groups	Parameters			
	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Lean control group	59.78 ± 1.78	53.62 ± 1.71	39.24 ± 1.07	14.21 ± 0.44
Obese control group	111.14 ± 1.85[a] a(85.92%)	81.15 ± 2.06[a] a(51.34%)	19.92 ± 0.56[a] a(-49.24%)	27.04 ± 0.64[a] a(90.29%)
Ob+*M. oleifera*	84.09 ± 1.89[bc] b(-24.34%) c(15.48%)	70.67 ± 1.19[bc] b(-12.91%) c(20.80%)	31.57 ± 0.93[b] b(58.48%) c(-2.26%)	19.60 ± 0.36[bc] b(-27.51%) c(17.01%)
Ob+Sim	72.82 ± 1.54[b] b(-34.48%)	58.50 ± 1.12[b] b(-27.91%)	32.30 ± 0.87[b] b(62.15%)	16.75 ± 0.29[b] b(-38.05%)

a: Significant change at P< 0.05 in comparison with the lean control group; b: Significant change at P< 0.05 in comparison with the obese control group; c: Significant change at P< 0.05 in comparison with the Simvastatin treated group.

The data illustrated in Table 3 represented the effect of treatment with *Moringa oleifera* ethanolic extract on pro-oxidants serum levels of obese female rats. The results revealed that serum lipid peroxide represented by malondialdehyde (MDA) and serum nitric oxide (NO) levels display significant increase (P<0.05) in obese group versus the lean control group. On the other hand, treatment of obese group with *Moringa oleifera* or with Simvastatin reverted this increase as indicated by the significant decrease (P<0.05) in serum MDA and NO levels as compared with those recorded in the obese control group (Table 3). Noteworthy, there are no significant changes (P>0.05) in serum MDA and NO levels between obese group treated with *Moringa oleifera* ethanolic extract and the obese group treated with Simvastatin (Table 3).

Table 3: Effect of treatment with ethanolic extract of *Moringa oleifera* on lipid peroxidation (MDA) and nitric oxide (NO) levels of female obese rats. Data were represented as Mean ± S.E of 8 rats/group.

Groups	Parameters	
	MDA (nmol/ml)	NO (µmol/L)
Lean control group	32.52±2.01	9.76 ± 0.72
Obese control group	70.46±3.11[a] a (116.67%)	47.75 ± 3.82[a] a(389.24%)
Ob+*M. oleifera*	43..96±2.09[b] b (-37.61%) c (4.09%)	19.52 ± 1.23[b] b(-59.12%) c (19.61%)
Ob+Sim	42.23±1.21[b] b (-40.07%)	16.32 ± 1.42[b] b(-65.82%)

a: Significant change at P< 0.05 in comparison with control group; b: Significant change at P< 0.05 in comparison with obese group.

Concerning serum leptin and resistin levels, obese group exhibited significant elevation (P<0.05) with respect to the lean control group. On the other side, serum leptin and resistin levels displayed significant depletion (p<0.05) in obese group treated with ethanolic extract of *Moringa oleifera* or Simvastatin when compared to the obese control group (Table 4). The opposite was observed regarding serum adiponectin level which showed significant reduction (P<0.05) in obese group in comparison with the lean control group. While, significant elevation (P<0.05) in serum adiponectin level was detected in obese groups treated with the ethanolic extract of *Moringa oleifera* or Simvastatin when compared to the obese control group (Table 4).

Table 4: Effect of treatment with ethanolic extracts of *Moringa oleifera* on serum leptin, adiponectin and resistin levels of female obese rats. Data were represented as Mean ± S.E of 8 rats/group.

Groups	Parameters		
	Leptin (Pg/mL)	Adiponetin (ng/mL)	Resistin (Pg/mL)
Lean control group	330.12 ± 2.91	11.23 ± 0.28	32.55 ± 0.42
Obese control group	753.31 ± 4.32[a] a(128.19%)	6.90 ± 0.091[a] a(-38.56%)	50.82 ± 0.95[a] a(56.13%)
Ob+*M. oleifera*	394.87 ± 7.51[b] b(-47.58%) c(3.95%)	9.06 ± 0.0065[bc] b(31.31%) c(-7.74%)	41.86 ± 0.77[bc] b(-17.63%) c(9.87%)
Ob+Sim	379.85 ± 9.84[b] b(-49.58%)	9.82 ± 0.088[b] b(42.42%)	38.10 ± 1.29[b] b(-25.03%)

a: Significant change at P< 0.05 in comparison with the lean control group; b: Significant change at P< 0.05 in comparison with the obese control group; c: Significant change at P< 0.05 in comparison with Simvastatin treated group.

Noteworthy, there is insignificant change (P>0.05) in serum leptin level between the group of rats treated with *Moringa oleifera* ethanolic extract and the group of rats treated with Simvastatin. However, significant decrease (P<0.05) in serum adiponectin level was demonstrated in obese group treated with *Moringa oleifera* ethanolic extract relative to obese group treated with Simvastatin. Moreover, significant increase (P<0.05) in serum resistin level was detected upon treatment of obese group with

Moringa oleifera ethanolic extract in comparison with the obese group treated with Simvastatin (Table 4).

DISSCUSION

A growing body of evidence indicates that natural products having anti-obesity effects can be arranged into five categories based on their distinct mechanisms; they produce (1) decreased lipid absorption, (2) decreased energy intake, (3) increased energy expenditure, (4) decreased pre-adipocyte differentiation and proliferation, or (5) decreased lipogenesis and increased lipolysis.[36]

The present study aimed at exploring the potential role of the ethanolic extract of *Moringa oleifera* in comparison with the anti-hypercholesterolemic drug Simvastatin in ameliorating the anthropometric measurements, hyperlipidemia, oxidative stress, serum leptin, resistin and adiponectin levels in obese rats.

In view of our data, both TC and AC showed significant increase in obese group. As well, BMI revealed significant increase in obese group relative to the lean control group. These findings come in line with the previously reported data that there is fat accumulation in the thoracic and abdominal regions due to the high cholesterol diet.[27] This indicates that the observed increase in body weight may be due to excessive energy intake and the adipose tissue accumulation. BMI has been stated to be a simple reliable estimate of body fat and obesity in rats.[27] As there are positive correlations between daily lipid intake and BMI as well as fat deposition.[37]

Treatment of obese groups with the ethanolic extract of *Moringa oleifera* or with the anti-hypercholesterolemic drug Simvastatin, resulted in a significant reduction in both TC and AC as well as BMI in comparison with the obese group control group. The observed reduction in these anthropometric measures in obese rats as a result of treatment with ethanolic extract of *Moringa oleifera* may be due to the inhibition of dietary lipid utilization. Earlier report by Dongmeza and co-workers indicated that a higher inclusion level of moringa extract or its fractions such as Saponins and tannins have been associated with the reduced energy required for protein and lipid biosynthesis leading to lower growth performance and nutrient utilization. Therefore, moringa has the ability to reduce body lipid and consequently energy retention.[38]

The recorded reduction in the anthropometric parameters in obese rats treated with Simvastatin may be attributed to the role of Simvastatin in altering adiponectin levels independent of adiposity.[39] Simvastatin has an effect on atherogenic lipoproteins overall, with a reduction in both LDL and triglyceride-rich lipoproteins, which together compose non-HDL cholesterol. Higher doses of Simvastatin have even shown to have greater effects on reducing non-HDL cholesterol and increasing HDL-C.[40]

The current results revealed significant increase in serum cholesterol, triglycerides and LDL levels accompanied with significant increase in serum HDL level in obese group with respect to the lean control group. Our results are in accordance with Son et al.[41] who detected hypercholesterolemia and hypertriglyceridemia in obese rats. More in detail, Fruchart et al.[42] have reported that lipids in adipose tissue are largely derived from circulating triglycerides especially during high-cholesterol diet feeding. The increased serum LDL level in obese rats has been also recorded in high cholesterol diet supplemented rats.[27] This event was explained by the decreased HDL level, as recorded in our study, thus decreasing the reverse cholesterol transport from the blood stream to the liver.[43] In addition, high cholesterol diet causes the oxidative stress leading to the increased production of reactive oxygen species (ROS). An increasing scientific literature provided ample direct and indirect evidence that the overproduction of ROS can induce cellular damage *via* oxidation of critical cellular components such as membrane lipids, proteins, and DNA. So, elevated level of blood cholesterol especially LDL is a known major risk factor for high cholesterol diet.[28]

Most international and national lipid treatment guidelines consider LDL-C the primary goal of hypolipidemic therapy. Ethanolic extract of *Moringa oleifera* or simvastatin, used in the present study could reduce serum cholesterol, triglycerides and LDL levels while increase serum HDL level in obese group relative to obese control group. These findings indicated that *Moringa oleifera* extract has beneficial effect on lipid profile through cholesterol reducing effect. Hassarajani et al.[44] have investigated its mechanism of action. Cholesterol homeostasis is maintained by the two processes, cholesterol biosynthesis in which HMG-Co-A reductase catalyzes rate limiting process and cholesterol absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion. The HMG-Co-A/mevalonate ratio has an inverse relationship to the activity HMG-Co-A reductase. The result of Jain et al.[28] indicated that the activity of this enzyme is significantly depressed by the ethanolic extract of *Moringa oleifera.* Thus, the cholesterol reducing action of the ethanol extract of *Moringa oleifera* indicated its hypolipidemic activity. *Moringa oleifera* leaf is a good source of phytochemicals including flavonoids, phenolics, carotenoids and β-sitosterol.[45] Saluja et al.[46] could isolate β-sitosterol from the stem of a hybrid variety of *Moringa oleifera.* β -sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain. It is believed that this compound has the ability to lower cholesterol by lowering plasma concentrations of LDL-C.[47] Therefore β-sitosterol in the leaves of *Moringa oleifera* is a bioactive phytoconstituents that accounts for the hypolipidemic influence of *Moringa oleifera* extract. Moreover, Moringa leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids.[45] Therefore, *Moringa oleifera* could prevent

the oxidization of LDL-C with consequent increase in HDL-C level.[48]

Simvastatin drug improve lipid profile *via*, lowering serum total cholesterol, triglycerides and LDL cholesterol concentrations and elevating serum HDL level as compared to the obese control group. Simvastatin belongs to HMG-CoA reductase inhibitors; a class of lipid lowering drugs. Our results are in conformity with ealier studies of Matikainen et al.[49] and Yao et al.[50]. Also, Delbosc et al.[51] reported that Simvastatin improves the lipid profile and increase HDL serum level through an improvement lipid dysfunction of obese rats and retard development of obesity complications.

Lipid peroxidation product (MDA) serum level recorded significant elevation in obese group when compared with the lean control one as shown in the present results. This result comes in line with that of Prasanna and Purnima[52]. The increased serum MDA level in obese rats could be explained by the increased caloric intake which represents an important factor in decreasing the mitochondrial membrane fluidity and increasing the generation of ROS.[53] As well, the study of Novelli et al.[27] revealed that there is a positive correlation between BMI and lipid peroxidation product concentration.

The significant reduction in serum MDA level observed in obese group treated with *Moringa oleifera* extract could be attributed to that the *Moringa oleifera* leaf extract contains polyphenols, therefore the free radical scavenging activity of the leaf extract may depend on its phenolic components.[54] Polyphenols have been known to possess powerful antioxidant activity *in vitro*. They inhibit lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, and can protect LDL from oxidation.[48] This mechanism is the main suggested mechanism that is responsible for the depletion of oxidative stress and lipid peroxidation product (MDA) serum level as a result of treatment of obese rats with *Moringa oleifera* extract.

The current results recorded significant depletion in serum MDA level in the obese group treated with Simvastatin, this could be attributed to its hypolipidemic effect and also to its antioxidant activity through lowering lipid peroxide formation.[55] Moreover, Sabri et al.[56] proved that Simvastatin markedly reduces the oxidative stress that is a contributory factor for obesity. It markedly reduced the production of toxic oxygen radicals thus reducing the oxidative stress independent of its cholesterol lowering effects.

The results of the present study revealed significant increase in the serum level of NO in obese group as compared to the lean control group. This could be attributed to the expression of endothelial NO synthase (eNOS) in subcutaneous adipose tissues.[57]

On the other hand, the obese group treated with *Moringa oleifera* extract showed significant reduction in serum NO level as compared to the obese control group. This finding could be attributed to the bioactive phenolic glycoside, namely 4-[(2′-O-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate (RBITC), which has been found to suppress nitric oxide synthase expression and nitric oxide (NO) production due to its antioxidant activity.[58]

The current study recorded that the treatment of obese group with Simvastatin induces significant decrease in serum NO level as compared to the obese control group. This result might be attributed to the ability of Simvastatin to decrease cytokine-stimulated NOS expression, independent on the cholesterol levels.[59]

Serum leptin level showed significant increase in obese group relative to the lean control group. Obesity increases caloric intake which represents an important factor in decreasing the mitochondrial membrane fluidity and increasing the generation of ROS.[60] Leptin, an adipocyte-derived satiety hormone, plays a crucial role in the regulation of food intake and energy expenditure through acting on its receptor expressed mainly in the hypothalamus.[61] There is a growing body of evidence indicating that leptin plays a role in fat metabolism and correlates with insulin resistance and other markers of the metabolic syndrome, independent on total adiposity.[62] Leptin is a cytokine like polypeptide produced by the adipocytes and it is overproduced during obesity due to the generation of ROS.[63]

In the present study, the treatment of obese group with the ethanolic extract of *Moringa oleifera* elecited significant decrease in serum leptin level. Moringa leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids.[45] Therefore, *Moringa oleifera* has the ability to scavenge free radicals with consequent inhibition of leptin level in serum as there is a significant positive correlation between leptin concentration and ROS generation.[64]

In this study, the treatment of obese group with Simvastatin produced significant decrease in serum leptin level. Changes in leptin serum levels are generally related to modifications in body weight or insulin sensitivity. Additionally, some experimental *in vitro* and *in vivo* evidences indicated that Simvastatin can inhibit leptin release from adipose tissue.[65]

In the present study, there was a significant decrease in serum adiponectin level in obese group with respect to that in the lean control group. Adiponectin is an adipocyte-secreted protein that circulates at high concentration.[66] Levels of adiponectin are reduced in obesity, and the suppression correlates with insulin resistance in obesity and related disorders.[67] Replacement of deficient adiponectin has a variety of salutary effects, including reducing glucose and lipid levels, lipid oxidation rates, and reducing vascular thickening.[68] Maeda et al.[65] found that adiponectin has disturbances in fatty acid catabolism and elevated serum TNF-α when challenged with high fat/high sucrose diet, associated with an increase in insulin resistance and

glucose levels. Accumulated evidences have demonstrated that obesity is associated with chronic inflammation and that both obesity and inflammation favor insulin resistance.[69] Adipose tissue produces adipocytokines, including leptin, tumor necrosis factor alpha (TNF-α), interleukins and adiponectin.[70] Activation of the TNF-α system has been associated with insulin resistance through the generation of defects in the phosphorylation of the receptor and decreasing the expression of insulin-sensitive glucose transporters.[65]

The current findings revealed that the treatment of obese group with the ethanolic extract of *Moringa oleifera* produced significant increase in serum adiponectin level in comparison with the obese control group. *Moringa oleifera* possesses anti-inflammatory capacity and it can inhibit the level of TNF-α.[71] This may be due to presence of the anti-inflammatory compounds in *Moringa oleifera* namely 4-[(2′-O-acetyl-α-l-rhamnosyloxy) benzyl] isothiocyanate, 4-[(3′-O-acetyl-α-l-rhamnosyloxy)benzyl] isothiocyanate and S-methyl-N-{4-[(α-l-rhamnosyloxy) benzyl]} thiocarbamate.[72] Thus, *Moringa oleifera* extract may elecit an improvement in the adiponectin serum level in obese rats *via* inhibition the TNF-α level.

Treatment of obese group with Simvastatin recorded significant increase in adiponectin serum level as compared to the obese control group. Simvastatin drug improved lipid profiles *via* its cholesterol lowering effect and elevation of HDL level.[51] Adiponectin concentrations have been found to be positively correlated with high-density lipoprotein-cholesterol.[73] By this way, Simvastatin could improve the level of adiponectin in obese rats.

In the current study, our results recorded a significant increase in serum resistin level in obese group versus the lean control group. Resistin is a member of a class of cysteine-rich proteins collectively termed resistin-like molecules. Resistin has been implicated in the pathogenesis of obesity-mediated insulin resistance. Resistin, like many other adipocytokines, may possess a dual role in contributing to disease risk.[74] Our findings could be attributed to that obesity is known to increase the release of several cytokines and other cellular mediators, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6.[70] It is possible that obesity induces resistin expression in monocytes. Resistin is considered as a link in the well-known association between inflammation and insulin resistance.[75]

Our data recorded significant depletion in the serum level of resistin in the obese group treated with ethanolic extract of *Moringa oleifera.* This could be explained by the anti-inflammatory effect of the active ingredients of *Moringa oleifera* which is responsible for inhibition of adipocytokines levels.[72] In this way, the ethanolic extract of *Moringa oleifera* could improve serum resistin level of obese rats.

The present study demonstrated significant decline in the resistin level in the serum of rats treated with Simvastatin. In accordance with our results, Zhao et al.[76] recorded that simvastatin reduce the expression and secretion of TNF-α in primary adipocytes isolated from hypercholesterolemia rabbits. This suggested that statins have direct anti-inflammatory effects on adipocyte. Furthermore, Wu et al.[77] stated that Simvastatin treatment significantly suppresses the oxidized-LDL. Furthermore, it has been found that the oxidized-LDL-induced mRNA expression and secretion of TNF- α and MCP-1 are also markedly inhibited by Simvastatin treatment. By this mechanism, Simvastatin could reduce resistin level in the serum of Simvastatin treated group.

CONCLUSION

In conclusion, the present study provided experimental evidences for the anti-obesity activity of *Moringa oleifera.* This effect was documented by the improvement of anthropometric measures, amelioration of the dyslipidemia, oxidative stress, hyperleptinemia, hypoadiponectinemia, and hyperresistinemia. The anti-obesity infleunce of *Moringa oleifera* could be attributed to its hypolipidemic, antioxidant and anti-inflammatory capacity.

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