**INTRODUCTION**

Obesity is considered a major public health problem worldwide, including Kingdom of Saudi Arabia (KSA), as approximately 12% of the world’s population suffer from obesity [1]. The danger of obesity is that increase in body weight leads to dyslipidemia, atherosclerosis, hyperten-
sion, insulin resistance and diabetes, what is referred to as metabolic syndrome [2]. The main reason for increased morbidity and mortality in people suffering from obesity is that most of the major risk factors for coronary artery disease coexist, and this condition may lead to premature cardiovascular disorders (CVD) [3].

To investigate the effectiveness of drug in obesity, numbers of experimentally induced obesity models are well established. However, diet-induced obesity is the most significant experimental model that signifies human obesity [4].

Hyperlipidemia-induced oxidative stress showed a critical role in the events of endothelial dysfunction and inflammation in CVD [5]. The literature suggests the relation between high fat induced obesity and endothelial dysfunction can be prevented by acute administration of ascorbic acid, proposing a role of ROS in obesity-related endothelial dysfunction [6,7].

Modern pharmacological therapies for the treatment of dyslipidemia and CVDs are available but expensive and also reported to have serious side effects resulting in patient non-compliance. Therefore, there is need to find alternative therapies principally from herbal sources because compare to synthetic medicine they are cheap and having least side effects. A number of plant extracts and their isolated compounds have been reported to maintain vascular endothelium in the animals fed with HFD [8,9].

Moringa oleifera Lam. (family Moringaceae) is a minerals and vitamin rich, nutritious and medicinally important tree species because a number of essential phytochemicals present in its different parts like leaves, pods and seeds [10,11]. Indeed, according to literature survey it can have 7, 10, 17, 9, 15 and 25 times more vitamin C, vitamin A, calcium, protein, potassium and iron than oranges, carrots, milk, yoghurt, bananas and spinach, respectively [12]. This plant has been honored as “Botanical of the Year” by the National Institute of Health (NIH). Previous studies have reported potent pharmacological effects of Moringa [13,14].

Many studies reported the therapeutic role of Moringa in hypercholesterolemia and hypertension, but there is lack of available data justifying its role in protecting vascular endothelium damage in rats induced by HFD. Therefore, this experiment was designed to investigate the ameliorative effect of Moringa oleifera alcoholic extract on chronic HFD-induced obesity related vascular dysfunction in Wistar albino rats.

EXPERIMENTAL

Reagents and chemicals

Acetylcholine chloride, phenylephrine (PE) and diagnostic kits for the estimation of serum total cholesterol (TC), TG, and HDL were purchased from Sigma (USA). The salts for physiological Krebs solution; calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride and sodium dihydrogen phosphate were purchased from E. Merck KGaA (Darmstadt, Germany). Krebs solution was freshly prepared in distilled water on the day of experiment.

Animals

Twenty-four adult healthy male and female Wistar albino rats (150 - 180 g) were used. The experimental protocol was approved by the Institutional Animal Ethics Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University, Kingdom of Saudi Arabia (approval no. BERC-001-10-18). The animals were acclimatized for one week before treatment and kept under standard laboratory conditions, in a ventilated room at 25 ± 2 °C, under a 12 h light/12 h dark cycle, with feed and water ad libitum.

Plant extract preparation

The fresh leaves of Moringa oleifera were harvested in the South West of Saudi Arabia (Jazan) during the summer season of 2017. Following harvesting, leaves were cleaned, dried under shade and pulverized using grinder. Using Soxhlet apparatus with methanol, pulverized leaves powder (500 g) was extracted. Following filtration, the extract was evaporated until dryness under reduced pressure. The extract was then stored in the refrigerator (2-4°C) for further use, and required concentrations were prepared immediately before use.

Preparation of high-fat diet

High fat diet was prepared as described by Jakobsdottir et al [15] with some modifications. Briefly, normal pellet diet i.e. standard diet (SD, 73 %) was ground and mixed thoroughly with cholesterol powder (1 % w/w), tallow (10 % w/w), egg yolk powder (10 % w/w), milk powder (6 % w/w). Tallow was used to induce obesity in HFD, as shown in Table 1. The resultant mixture was
mixed with water and made into pellets which were then oven-baked for proper drying to avoid fungal contamination.

### Table 1: Composition of high fat diet (HFD)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Normal Pellet Diet (NPD)</td>
<td>73</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1</td>
</tr>
<tr>
<td>Tallow</td>
<td>10</td>
</tr>
<tr>
<td>Egg</td>
<td>10</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>6</td>
</tr>
</tbody>
</table>

#### Experimental design

Animals were randomly divided into 4 experimental groups (6 animals in each). Group I served as standard diet (SD) group and rats were fed with standard pellet diet for 12 weeks. Group II served as high fat diet (HFD) group and rats were fed with HFD for 12 weeks. Group III and IV rats administered with HFD plus oral administration of two increasing doses of MEMO at 200 and 400 mg/kg/day, respectively.

#### Evaluation of MEMO effect on body and organ weights

To investigate the effect of MEMO on body weight (BW), liver weight (LW), and retroperitoneal fat pad weight (RFPW), two doses of MEMO (200 and 400 mg/kg/day) were given orally for three weeks to HFD fed rats (9 weeks on HFD prior to MEMO administration).

#### Evaluation of the effect of MEMO on food intake

The average daily feed intake of all the animals was recorded according to the method of Diniz et al [16]. Feed intake was measured every day by weighing the amount of food put into the food tray and that remaining in them.

#### Evaluation of the effect of MEMO on waist, Lee index and BMI

Waist, Lee index and BMI of the all the rats in different groups were determined according to a previously reported method [17].

#### Evaluation of MEMO effect on lipid profile

Blood samples, collected in suitable centrifuge tubes from orbital sinus under light ether anesthesia using glass capillary were centrifuged (3,000 rpm) at 4 °C for 20 min and separated serum were stored at -80 °C for lipid profiling studies. Serum concentrations of total cholesterol (TC), Triglycerides (TG) and HDL, were analyzed using diagnostic kits as described in the instructions of the manufacturer (Sigma–Aldrich Chemical Co., USA). VLDL and LDL were calculated as in Eqs 1 and 2 [18].

\[
\text{VLDL} = \frac{\text{TGs}}{5} \quad \text{(1)}
\]

\[
\text{LDL} = \text{TC} - (\text{VLDL} + \text{HDL}) \quad \text{(2)}
\]

#### Measurement of vascular endothelial dysfunction

At the end of the experiment, all animals SD, HFD and MEMO (200 and 400 mg/kg/day) were fasted for 16 h and sacrificed by blow on head. After opening the abdomen and thoracic cage, their aortae were carefully isolated and kept in physiological Krebs solution with the following composition in mM: NaCl: 118.4, KCl: 4.7, CaCl<sub>2</sub>: 2.5, KH<sub>2</sub>PO<sub>4</sub>: 1.2, MgSO<sub>4</sub>: 1.2, NaHCO<sub>3</sub>: 25 and glucose: 11) aerated with oxygen. After cleaning from the adjacent tissues, aorta was cut into wide rings (2-3 mm length). Individual aortic rings were evaluated for endothelial reactivity using isolated EmkaBath (France) filled with Krebs solution (37 °C), bubbled with oxygen and connected to a computer-based software (IOX2) to record isometric tension. Aortic rings were allowed to equilibrate in organ bath for 45 – 60 min, at a resting tension of 2 g with replacement of fresh Krebs solution every 15 min. After the stabilization of the isometric tension, inhibitory concentration–response curves (CRCS) of ACh were prepared against PE (1 × 10<sup>-6</sup> M)-mediated spasm [19].

#### Histological examination of aorta

At the end of the experiment, thoracic aortae were isolated and fixed in 4% formaldehyde and processed routinely for paraffin embedding. Aortae fixed tissues slices of 5 μm thickness were obtained with rotary microtome. General structure of the aortae of all group of animals were investigated using hematoxylin and eosin (H & E) stain. Collagen fibers and elastic fibers were investigated using van Gieson and Verhöeff’s stain, respectively. Stained sections were examined under a light microscope (Hund Wetzlar H600/12, Germany, fitted with digital camera, Canon EOS 550D).

#### Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). To evaluate intergroup variability, data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test using GraphPad Prism 7.0 (GraphPad Software, Inc, USA). For all
comparisons, statistical significance was defined as $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$.

RESULTS

Effect of MEMO on body and organ weights

Animals feed with HFD resulted in significantly increased in body weight, LW/BW and RFPW/BW ratio when compared with rats feed with standard diet (SD) ($p < 0.001$). However, body weight, LW/BW and RFPW/BW ratio was found to be significantly decreased in HFD-induced obese rats treated with both MEMO doses, in dose dependent manner (Figure 1). Subsequently, the gaining of body weight in MEMO treated rats (at weeks 10, 11, and 12) was found to be significantly decreased compared to HFD-induced obese rats (Figure 2 and Figure 3).

Effect of MEMO on waist, Lee index and BMI

Waist, Lee index and BMI of rats was found to be significantly elevated after 9 weeks of HFD treatment when compared with SD group, irrespective of sex (Figure 4). However, MEMO administration to the HFD-induced obese rats for 3 weeks at both doses (200 and 400 mg/kg/day) resulted in significant reductions in waist, Lee index and BMI.

Effect of MEMO on food intake

The average daily feed intake of all rats was found to be same at the beginning of the experiment. However, HFD treatment for 9 weeks resulted non-significant increase in feed intake (Figure 5) as compared to SD rats. Both MEMO treatments for 3 weeks in HFD fed rats significantly decreased feed intake when compared to the HFD fed rats.
As depicted in Table 2, rats fed with HFD for 9 weeks resulted in a significant increase in the levels of TC, TG, LDL-C ($P < 0.001$), VLDL-C, and a significant decreased in levels of HDL-C ($P < 0.001$) when compared to SD feed rats. However, MEMO administration for three weeks significantly reduced the hyperlipidemic effect of HFD ($P < 0.001$) in dose dependent manner.

Effect of MEMO on histopathological changes

Photomicrographs of aorta of HFD-induced obese rats showed presence of fat accumulation in form of adipocytes (arrow at photo 2A x35), weakness of elastic fibers compared to both normal and treated groups. Increased collagen elastic ratio indicated by increased collagen layer (arrow points at red color in photo 2B x 30). Treatment of MEMO at a dose of 400 mg/kg/day resulted in improvement and photomicrographs showed almost normal architecture of aorta regarding all aspects of general features, collagen elastic ratio and elastic fibers status (Figure 7).

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
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<tbody>
<tr>
<td>SD</td>
<td>152.95±10.45</td>
<td>22.91±2.72</td>
<td>83.98±3.84</td>
<td>4.76±0.41</td>
<td>64.20±9.55</td>
</tr>
<tr>
<td>HFD</td>
<td>375.26±16.30***</td>
<td>85.41±5.99***</td>
<td>47.91±1.28***</td>
<td>17.08±1.19***</td>
<td>310.27±17.46***</td>
</tr>
<tr>
<td>HFD + MEMO 200</td>
<td>268.81±9.93**</td>
<td>70.71±5.81 ns</td>
<td>52.34±6.43 ns</td>
<td>14.14±1.16 ns</td>
<td>202.32±8.30 ns</td>
</tr>
<tr>
<td>HFD + MEMO 400</td>
<td>206.18±9.68</td>
<td>41.67±3.07***</td>
<td>66.66±3.99</td>
<td>8.33±0.61 ns</td>
<td>131.85±10.80 ***</td>
</tr>
</tbody>
</table>

**$P < 0.001$, compared with SD (Student t test), ***$p > 0.05$, **$p < 0.05$, ***$p < 0.01$, ****$p < 0.001$, when compared with HFD (one-way ANOVA followed by post Tukey’s test)
DISCUSSION

It is well known that obesity associated with increased cardiovascular risk, even though, there is no effective treatment available for the obesity and obesity related vascular complications. Therefore, the present experiment was designed to evaluate the medicinal use of M. oleifera leaves in obesity-induced dyslipidemia and vascular endothelial dysfunction. The medicinal use of MEMO was investigated at doses of 200 and 400 mg/kg/day for 3 weeks against HFD-induced dyslipidemia and related disorders such as vascular endothelial dysfunction. High fat diet induced significant differences in adiposity when compared with SD groups, confirming the experimental model. Obesity as a result of gain in the body weight is an evident effect of HFD intake [20]. Wistar albino rats fed with HFD for 12 weeks resulted in significant increase in body weight, LW/BW, RFPW/BW, waist, Lee index, BMI and food intake when compared with rats fed with SD only. On the other hand, MEMO treatment at both doses for 3 weeks in HFD-induced obese rats produced a significant decrease in body weight, LW/BW, RFPW/BW, waist, Lee index and BMI, and also decreased the amount of food consumed daily by rats when compared with HFD group. It is important to report that in addition to the beneficial effects of MEMO in hypertension [21] and dyslipidemia, it also significantly reduced the body weight comparable to the SD fed rats, via decreasing the diet intake, thus confirming the weight-reducing potential of MEMO.

It has been reported earlier that HFD-induced dyslipidemia model resulted in significant increase in serum total cholesterol (TC), LDL and decreased HDL levels by improving the intestinal absorption and secretion, and decreasing cholesterol metabolism [22]. In the present study also, HFD treatment resulted in significant increase TC, TG, VLDL, and LDL levels while decreased HDL levels whereas, rats treated with MEMO at both tested doses significantly decreased the TC, TG, VLDL, and LDL levels while increased HDL levels similar to animals feed with SD. Administration of MEMO significantly improve the lipid profile in HFD treated rats, may be due to the presence of phytochemical constituents like flavonoids and saponins in the plant [23].

It has been reported earlier that flavonoids lower the LDL levels and increases the HDL concentrations in hypercholesteremic animals [24]. Saponins act by inhibiting pancreatic lipase activity in HFD fed animals leading to larger fat excretion due to the reduced intestinal absorption of dietary fats [25]. High fat diet also resulted in induction of oxidative stress in rats and causes increased oxidation of LDL which plays an important role in atherosclerosis. Therefore, antioxidants are best option in preventing cellular damage caused by oxidative stress [26]. Previous literature reported the presence of strong antioxidants in the Moringa [21] thus offer further benefits against oxidative stress caused by hypercholesterolemia.

Obesity is also associated with vascular endothelial dysfunction may be caused by a range of metabolic disorders as the atherosclerosis, hypertension, dyslipidemia and diabetes, which are associated with vascular oxidative stress [27]. Many scientific reports have confirmed the relationship of hypercholesterolemia with impaired endothelium-dependent relaxation in atherosclerotic coronary arteries and angiographically smooth coronary arteries [28,29]. Various vasoactive substances are synthesized and released by the endothelium to regulate peripheral vascular resistance. Vascular endothelial dysfunction induced by HFD has been suggested to be caused by various factors, such as hypertension [30], hypertriglyceridemia [31] and excess production of oxidants and/or deficiency of antioxidant systems [32].

In present study on HFD-mediated dyslipidemic rats, treatment with MEMO caused reversal of the endothelial dysfunction by improving the acetylcholine-mediated aortic tissues laxation, whereas HFD rat's aorta did not show vasorelaxation. This shows that MEMO treatment protected vascular endothelium from harmful effects of HFD. Endothelial dysfunction provides a reasonable pathophysiologic explanation for the deleterious effects of various risk factors on coronary artery disease. Obesity can also result in vascular dysfunction, including the aorta, leading to alteration in the vascular histology. In this study, aorta of HFD fed rats showed presence of fat accumulation in form of adipocytes, weakness of elastic fibers layers, increased collagen elastic ratio indicated by increased collagen layer when compared to aorta of SD fed rats.

Previous studies reported that increased BMI is usually associated with stiffening and increased arterial wall thickness [33]. The findings reported in this study corroborate previous findings on BMI as an important predictor of increased cardiovascular mortality. However, MEMO treatment at dose of 400 mg/kg/day significantly restored the integrity of aorta, and resulted in better improvement as showed by almost normal
architecture of aorta regarding all aspects of general features, collagen elastic ratio and elastic fibers status. These findings suggest that treatment with MEMO restores vascular structural and functional integrity and histopathological alterations in obesity.

CONCLUSION

The antihyperlipidemic and endothelial cell-protecting potential of MEMO have been found in this study. This plant material improves both lipid profile and vessel endothelial integrity. Thus, the findings of this study justify the traditional medicine use of *M. oleifera* leaves in the treatment of dyslipidemia and related cardiovascular disorders. However, there is still need to perform further studies to isolate and identify the active compounds of *M. oleifera* in order to confirm their therapeutic activity.

DECLARATIONS

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Conflict of interest

Authors declare that they have no conflict of interest with regard to this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article. Hassan A. Madkhali and Khalid M Alharthy designed the study. Hassan A. Madkhali, Mohammed Asiri, Majid A Ganaie and Mohd Nazam Ansari did experimental work, collected and analyzed the data. Najeeb Ur Rehman performed in vitro experiments for vascular endothelial dysfunction study. Abubaker M Hamad performed histopathology of aorta. Hassan A. Madkhali wrote the manuscript with support from Khalid M Alharthy, Majid A Ganaie, Mohd N Ansari and Najeeb Ur Rehman. All authors read and approved the manuscript for publication.

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REFERENCES


