

## Evaluating the Effect of *Moringa peregrina* (Forssk.) Fiori seeds on some Biochemical and Oxidative Stress Markers in Obese Experimental Rat

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### Abstract

Obesity is a primary reason for morbidity and mortality. The current study aimed to evaluate the anti-obesity and antioxidant effect of *Moringa peregrina* (MP) seeds oil in obesity-induced rats. Forty male Sprague Dawley rats were divided into four equal groups of 10 rats each. Group C (control); group OB rats were fed a high-fat diet (HFD). Group MPI: rats treated with HFD+MP seeds oil (250 mg/kg b.wt./day, orally) and group MPII. rats treated with HFD + MP seeds oil (500 mg/kg b.wt./day, orally). Blood samples were collected by the end of the experiment for determination of serum total cholesterol (TC), Triacylglycerols (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), and liver functions (ALT, AST, ALP). Moreover, malondialdehyde level (MDA), superoxide dismutase and catalase activity were measured in both plasma and liver. The results showed that TC, TAG, LDL, ALP, AST, ALT and MDA were significantly higher in obese rats than in the control group. On the other hand, administration of MP seeds oil to HFD-fed rats improved oxidative stress and lipid metabolism abnormalities by ameliorating TC, TAG and liver enzyme activities. These results suggest that MP seeds oil effectively enhances obesity-associated parameters and decreases its related complications.

**Keywords:** *Moringa peregrina*; oil, obesity; lipid profile; liver enzymes; MDA, SOD.

### Introduction

Obesity is an abnormal or excessive accumulation of fats in different parts of the human body. It is a chronic, complex, and multifactorial metabolic disorder that impairs

health (Saalbach and Anderegg, 2019). It occurs as a result of a combination between an individual's genetic and environmental factors as well as reduced physical activity and high-fat diet (O'Rahilly and Farooqi, 2000). Obesity is associated with the risk of developing diabetes mellitus, insulin resistance, cardiovascular diseases, musculoskeletal disorders,

hyperlipidemia and liver functions impairment and some cancers (**Wu and Ballantyne, 2020**).

Obesity is a significant public health problem that has progressively worsened over the last 5 decades and is considered the second cause of death after smoking (**Saalbach and Anderegg, 2019**).

To date, various strategies for treating obesity include lifestyle changes and drugs for reducing the absorption of lipids and/or changing their mobilization and utilization (**Kang and Park, 2012; Cheung et al., 2013**). The administration of anti-obesity drugs is restricted due to various clinical side effects such as dry mouth, insomnia, cardiovascular problems, anxiety, and constipation (**Yun, 2010; Cheung et al., 2013**). Accordingly, looking for natural products with lesser side effects has received much concern. Many medicinal plants and their phytochemical constitute showed promising efficacy to prevent, and/or treat obesity with safer side effects, cheaper cost and long-term effects (**Bessesen and Van Gaal, 2018**). In this regard, natural bioactive components in plants such as phenols and flavonoids have been reported to be efficient in controlling obesity (**Elsayed et al., 2015; Pan et al., 2016**). The most effective remedy to curb obesity is to take fewer fats, carbohydrates and meat and to keep an active lifestyle.

Moringa is one genus from family Moringaceae, which contains 13 different species. *Moringa peregrina* (MP) is domestic to the Arabian Peninsula (**Olson, 2002**), with a valuable source of many bioactive phytochemicals like phenolics, essential oils antioxidants, terpenoids and steroids (**Ferreira et al., 2008; Elsayed et al., 2015**). **Shamlan et al. (2021)** reported that MP leaves and seed oil enhance the anti-inflammatory effects. Also, the MP oil has important positive effects on arthritis and inflammation. According to **Alkudhayri et al. (2021)**, MP leaves extract exhibited potential anti-obesity as well as hepatoprotective effects in obese rats, by reducing absorption of lipids and increasing antioxidant activities in liver.

**Abou-Hashem et al. (2019)**, reported that Seed extract of MP showed a significant antitumor effect through inducing apoptosis in HELA and PC-3 cells. In a recent study, **Senthilkumar et al. (2020)** showed that MP essential oil contains 33 compounds by using Chromatography Spectrometry analyses such as

geijerene, linalool, caryophyllene oxide, n-hexadecane and carvacrol, represented by 33.38%, 23.36%, 19.28%, 12.59% and 1.89%, respectively. Thus, MP oil can be considered a choice for synthetic antioxidants. Although MP has a very important potential therapeutic value, experimental data of MP seeds oil in treating obesity is very rare, therefore the present study aimed to evaluate *Moringa peregrina* (Forssk.) Fiori seeds effects on some biochemical and oxidative stress markers in obese experimental rats.

## Materials and methods

### Plant material

Seeds of *Moringa 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 seed crushes with 1 L of solvent and left overnight. After evaporating the solvent, the residual 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 via dichloromethane/methanol (1:1, v/v) mixture (**Hasan et al., 2007**)

### Animals and Study design

Forty male Sprague Dawley rats, *Rattus norvegicus domestica*, (110-118 g) were procured and housed in Laboratory Animal House, King Saud University, Riyadh, Saudi Arabia. Rats were allowed to adapt and acclimatize by maintaining natural room temperature, and daylight cycle for two weeks and feeding by libitum control diets. For the whole experimental period, 20 weeks, rats were housed at a  $24 \pm 2$  C and humidity level  $60 \pm 5\%$ . The experimental methodology was carried out according to the National Institutes of Health for the Care and Use of Laboratory Animals guidelines (NIH Publication No. 8523, revised 1996)

The rats were divided randomly into four groups (n=10/ group) as follows: 1<sup>st</sup> Group (C) received a standard diet, 2<sup>nd</sup> group (OB) were fed with HFD as an obesity induction agent, 3<sup>rd</sup> group (MPI) received HFD + MP seeds oil extract (250 mg/kg bw/ day) and 4<sup>th</sup>

group (MPII) received HFD + MP seeds oil extract (500 mg/kg bw/ day). Obesity was induced over 12 weeks according to **Woods et al. (2003)** and MP seeds oil extracts were applied daily by intragastric administration for the last 8 weeks.

#### Dose selection

MP concentrations applied in the current study were chosen according to previous results where 200 mg/kg MP has protective effects (**Azim et al., 2017**). Moreover, MO was proven to have ameliorative effects against obesity and hyperlipidemia at different doses from 200, to 600 mg/kg (**Bais et al., 2014; Metwally et al., 2017**).

#### Serum, plasma and tissue sampling

By the end of the experimental period, all rats were euthanized under diethyl ether anaesthesia, blood samples were drawn from the heart directly using cardiac puncture methodology (3 ml/rat) and centrifuged for 20 min at 3000 rpm, finally, plasma samples and sera were harvested and stored at -20 for further biochemical analysis. To eradicate any erythrocytes or clots, liver samples were removed and weighed individually before being washed by phosphate-buffered saline (PBS), pH 7.4. A 10% homogenate from each tissue was prepared by taking a particular weight, minced and mixed with ice-cold 0.1 M phosphate buffer (pH 7.4). The lysates were then centrifuged at 1,500 ×g at 4°C, the obtained supernatants were kept at -80°C for evaluation of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). All parameters were determined using available commercial kits (Biodiagnostics Co.), and absorbance values of all samples and standards were measured by a UV spectrophotometer.

#### Statistical analysis

The data for all groups were tabulated and represented by Mean ± standard error (n = 9). For multiple comparisons, the Tukey-HSD test was used, while the Dunnett-two-sided test was applied to compare the control with other groups. XLSTAT program (Addinsoft, NY, USA) was used to perform all statistical analyses.

## Results

### Effects of *M. peregrina* seeds oil on body weight

Abnormal increment in body weight is the primary indicator of obesity development and for treatment monitoring. **Figure 1** shows the primary and end rats body weights in all groups. The final body weight of group OB (+ve control) increased significantly as compared to other groups (mean ±SD= 343.5 ±3.67 gm,  $p < 0.05$ ). supplementation of MP seeds oil in groups MPI and MPII decreased the final body weight (mean ±SD=312.5± 7.4 gm and 302.67± 4.22, respectively) but was still significantly higher than the control group (mean ±SD= 257.67 ±5.17 gm;  $p < 0.05$ ).

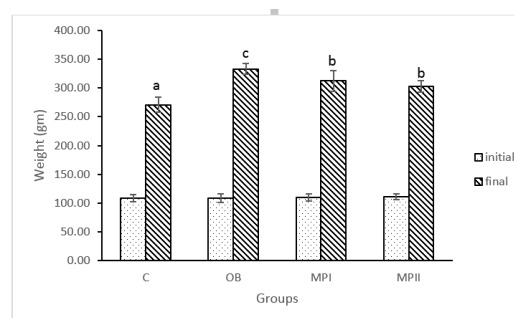


Figure 1: Means and standard deviation values (error bars) of initial and final body weight by groups. Different small letters indicate significant differences between groups. C= control without treatment, oil, OB= obese group, MPI= obese rats treated with 250 mg/kg bw and MPII= obese rats treated with 500 mg/kg bw.

### Effects of *M. peregrina* seeds oil on serum biochemical parameters

The obtained biochemical data are represented in **table 1**. Data show a significant increment in cholesterol, triglycerides, LDL, ALT, AST and ALP in the obesity-induced group (OB) as compared to all other groups.

On the other hand, HDL level was significantly lower in the obesity-induced group (OB) as compared to all other groups. While HDL level in the MPII group decreased significantly as compared to those in C group but not different from MPI group.

Table 1: Effect of MP seeds oil supplementation (250 and 500 mg/kg bw) on some serum cholesterol, triglycerides, LDL, HDL, AST, ALT and ALP

Parameters	Groups			
	C	OB	MPI	MPII
Cholesterol (mg/dl)	235.47±3.60 <sup>a</sup>	436.48±28.86 <sup>b</sup>	240.69±39.4 <sup>a</sup>	254.97±23.13 <sup>a</sup>
Triglycerides (mg/dl)	152.97±12.2 <sup>a</sup>	292.15±18.12 <sup>b</sup>	194.66±16.6 <sup>a</sup>	179.16±10.14 <sup>a</sup>
LDL (mg/dl)	66.80±2.37 <sup>a</sup>	219.35±13.98 <sup>b</sup>	95.07±6.13 <sup>a</sup>	68.79±3.97 <sup>a</sup>
HDL (mg/dl)	138.80±2.87 <sup>a</sup>	77.64±4.58 <sup>b</sup>	131.74±4.59 <sup>a d</sup>	115.93±2.87 <sup>d</sup>
ALT (U/ml)	25.05±1.12 <sup>a</sup>	60.69±3.29 <sup>b</sup>	24.02±2.64 <sup>a</sup>	24.60±1.64 <sup>a</sup>
AST (U/ml)	23.33±2.47 <sup>a</sup>	46.57±2.90 <sup>b</sup>	30.60±2.77 <sup>a</sup>	34.00±1.21 <sup>a</sup>
ALP (U/ml)	64.68±2.92 <sup>a</sup>	143.52±14.47 <sup>b</sup>	71.83±2.71 <sup>a</sup>	69.78±5.43 <sup>a</sup>

Data are presented as (Mean ± S. E). S.E = Standard error. Mean values with different superscript letters for the same parameter are significantly different at ( $p=0.05$ ).

#### Effects of *M. peregrina* seeds oil on SOD, MDA, and catalase in plasma and liver parameters

Superoxide dismutase activity in plasma and liver of all treated groups is shown in **figure 2**. In both plasma and liver, a significant decrease was found in OB group as compared to other groups.

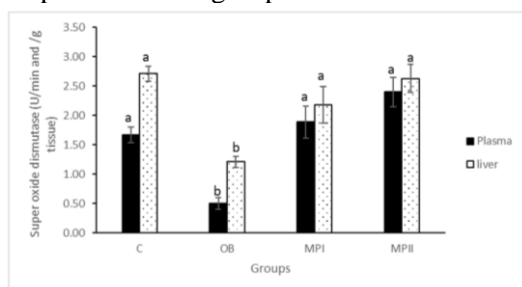


Figure 2: Superoxide dismutase activity (SOD) in plasma (U/min) and liver (U/min/gm tissue) of male Sprague Dawley rats in control, OB, and two groups treated with different doses of MP seeds oil (250 and 500 mg/kg bw). Bars represent means± SE, letters on each bar indicate significantly different groups

The effects of MP seeds oil on malondialdehyde levels in both plasma and liver are shown in **figure 3**. In plasma, MDA levels were significantly higher in OB group than the control, both doses of MP reduced MDA levels. Similarly, MDA concentrations in the liver exhibited a significant elevation in OB group as compared to all treated groups except

the MPII group. A dose of 250 mg/kg bw in the MPI group significantly normalized the MDA level in the liver and reduced it to its normal level in the control group.

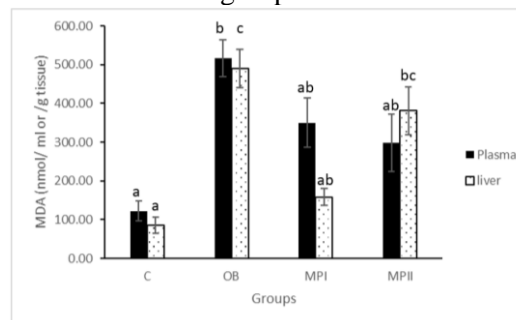


Figure 3: Malondialdehyde (MDA) in plasma and liver of male Sprague Dawley rats in control, OB, and two groups treated with different doses of MP seeds oil (250 and 500 mg/kg bw). Bars represent means± SE, letters on each bar indicate significantly different groups

The effects of administration of MP seeds oil on catalase activity in both plasma and liver are shown in **figure 4**. In plasma, catalase activity was significantly lower in the OB group as compared to all groups except the MPII group. Similarly, catalase activity in the liver showed significant low activity in OB group as compared to all treated groups.

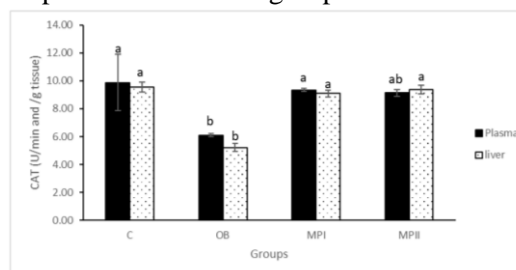


Figure 4: Catalase activity (CAT) in plasma (U/min) and liver (U/min/gm tissue) of male Sprague Dawley rats in control, OB, and two groups treated with different doses of MP seeds oil (250 and 500 mg/kg bw). letters on each bar indicate significantly different groups

## Discussion

Obesity is associated with many metabolic abnormalities which increase the risk of cardiovascular problems (Vecchione *et al.*, 2002). Moreover, obesity was proven to be a primary risk factor for several health issues, such as liver and spleen dysfunction (Chandrasekaran *et al.*, 2012).

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 (LD50) 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.

The current results showed a significant elevation in serum concentrations of cholesterol, triglycerides, LDL and MDA in the obesity-induced group, while HDL was significantly decreased in the same group. This result is in agreement with **Hussein et al. (2017)** who stated that HFD-fed mice showed a significant elevation in plasma cholesterol, triglycerides and low density lipoprotein which may cause abnormalities in metabolism of lipid (**Taboada et al., 2006**). Supplementation of MP seeds oil to HFD fed rats showed anti-obesity, hypolipidemic, antioxidant and hepatoprotective effects. Treating HFD-induced obesity with extract of seeds oil of MP (250 and 500 mg/ kg bw) decreased body weight, enhanced liver functions, and inhibited oxidative stress. These results are in agreement with **Alkudhayri et al. (2021)** where ethanolic extracts of MP leaves decreased body weight and fat gain, therefore preventing liver steatosis as well as improving antioxidative mechanisms.

The MP seeds oil also decreased both cholesterol and triglycerides in HFD-fed rats, an indicator of inhibition fat absorption in intestine. MP may decrease absorption of by inhibiting pancreatic lipase activity, which is comparable to action of orlistat (**Viner et al., 2010**). Moreover, similar results were obtained by administrating of MP leaves extract (**Alkudhayri et al., 2021**).

Liver is responsible for all metabolic processes in the body. Administration of MP enhanced liver functions of rats fed on HFD, as

shown by the normalization of ALT, AST, and ALP which are indicator of liver function.

Obesity is responsible of elevating oxidative stress by increasing free radicals production and impaired antioxidant capacity, especially suppression of antioxidant enzymes (**Othman et al., 2019**). The current study results show increased oxidative stress in both plasma and livers of rats fed an HFD indicated by a significant elevation in lipid peroxidation and low levels of antioxidants. These findings are in agreement with **Matsuzawa-Nagata et al. (2008)** who found that oxidative stress increased in HFD induced obesity in mice model.

MP administration normalized different antioxidants in both plasma and livers of obese rats. Superoxide dismutase (SOD) and catalase (CAT) are two main antioxidant enzymes which essential for the conversion of superoxide anions to hydrogen peroxide. SOD reduces blood glucose levels by removing superoxide radicals from plasma and the liver (**Zhang et al., 2015**). Treating the obese rats with two doses of MP resulted in higher SOD and CAT activities in both plasma and livers, which proves the capability of MP to eradicate ROS and protect the SOD/CAT system that removed superoxide radicals.

## Conclusion

The current study results showed that MP seeds oil administration to HFD-fed rats helped in improving dyslipidemia, oxidative stress and liver functions by enhancing lipid profile, MDA, and liver enzymes. It could be proposed that MP seeds oil has antiobesity and antioxidant properties and might be used as a medicine to control obesity and its pathophysiological implications.

## Declaration of Competing 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 paper.

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## الملخص العربي

### عنوان البحث: تقييم أثر بذور المورينجا بريجرينا (فورسك) علي بعض المعايير البيوكيميائية وعلامات وطأة الأكسدة علي السمنة المستحثة معمليا في الفئران

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تعد السمنة سبب رئيسي للأمراض والوفيات. تهدف الدراسة الحالية الي تقييم الاثر المضاد للسمنة ومضادات الأكسدة لمستخلص بذور المورينجا بريجرينا علي السمنة المستحثة معمليا في الفئران. تم تقسيم أربعون ذكر من فئران سبروج دويلي الي أربع مجموعات، تحتوي كل مجموعة علي عشرة فئران، علي النحو التالي المجموعة الضابطة (نظام غذائي طبيعي) ومجموعة السمنة (نظام غذائي عالي الدهون) و مجموعة مورينجا بريجرينا I: تم معالجتها أولا بنظام غذائي عالي الدهون ثم مستخلص بذور المورينجا بجرعة ٢٥٠ مجم/كجم من وزن الجسم والمجموعة الأخيرة مجموعة مورينجا بريجرينا II: تم معالجتها أولا بنظام غذائي عالي الدهون ثم مستخلص بذور المورينجا بجرعة ٥٠٠ مجم/كجم من وزن الجسم. بنهاية التجربة تم تجميع عينات الدم وتعيين تركيز كلا من الكوليسترول (TC) و الدهون الثلاثية (TAG) والبروتين الدهني عالي الكثافة (HDL) وكذلك البروتين الدهني منخفض الكثافة (LDL). بجانب ذلك تم أيضا تعيين الانزيمات الكبدية (ALT, AST, ALP) ومالون داي ألدهيد (MDA) ونشاط كلا من سوبر اكسيد ديسميوتيز (SOD) والكاتاليز (CAT). أشارات النتائج الي أن مجموعة السمنة اظهرت ارتفاع معنوي في تركيز كلا من الكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة و المالون داي ألدهيد وكذلك ارتفاع معنوي في نشاط انزيمات الكبد مقارنة بالمجموعة الضابطة. وعلي جانب اخر، أدت المعالجة بمستخلص بذور المورينجا الي تحسين معايير الأكسدة وأيض الدهون. تشير هذه النتائج الي أن مستخلص بذور مورينجا بريجرينا يعزز بشكل فعال العوامل المرتبطة بالسمنة ويقلل من المضاعفات المرتبطة بها.