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Effects of *Moringa oleifera* Lam. seed oil on lipid profiles and bone biomarkers in ovariectomized rats

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Abstract

This research evaluated effects of *Moringa oleifera* seed oil (MSO) on lipid profiles and bone biomarkers in ovariectomized (OVX) rats. To study the preventive effect and the recovery effect, rats were ovariectomized and fed with MSO on day 3 after ovariectomy (Group 1) and on day 60 after ovariectomy (Group 2), respectively. Each group was then divided into five subgroups; sham-operated control (SHAM); OVX rats treated with vehicle; OVX treated with MSO 0.25 mL/100g BW/day, OVX treated with MSO 0.50 mL/100g BW/day and OVX treated with 17β-estradiol 10 µg/kg BW/day. After 6 weeks of treatment; serum of rats in each treatment group was analyzed to determine lipid profiles [the total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)] and bone biomarkers [alkaline phosphatase (ALP), serum calcium, serum phosphorus]. The urine samples were also collected to determine urine calcium, phosphorus and creatinine. OVX rats treated with MSO showed significant decreases in LDL-C, increases in HDL-C compared to the OVX-control group. MSO treated rats restored the elevated serum levels of Ca, P and ALP. The oil also decreased urine Ca and P levels in all OVX groups compared to the SHAM group ($p < 0.05$). Taken together, these findings suggest that MSO intake can improve lipid profiles and bone biomarkers in ovariectomized rats.

Keywords: *Moringa oleifera* seed oil, Lipid profiles, Bone biomarker, Osteoporosis, Ovariectomized rat

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1. Introduction

After the onset of menopause, the risk of coronary heart disease (CHD) in women increases dramatically because of hormone deficiency especially estrogens [1]. Decreased ovarian function involved in increased plasma concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL-C), and an increase in LDL/HDL ratio are among the important risk factors for the development of CHD [2]. Many research groups have reported that estrogens are important regulators of lipid homeostasis. The beneficial effects of estrogen might decrease LDL-C and increase HDL-C production of neurotrophic growth factors, which modulate neuronal growth, survival and aging [3].

To date, the use of herbal medicine has become a common practice due to the presence of various bioactive phytochemicals in herbal plant. Numerous studies revealed that consumptions of some vegetable oil including soybean, rapeseed, flaxseed, and walnut oil are effective to reduce plasma lipid levels. Navin *et al.* [4] reported that virgin coconut oil has beneficial effect in lowering lipid components. The oil reduced total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C levels and increased HDL-C cholesterol in serum and tissues in rats. Flaxseed oil and sesame oil have also been reported to have benefits in reducing plasma cholesterol, LDL-C, and bone biomarkers induced by ovarian hormone deficiency in OVX rats [5]. Furthermore, pomegranate seed oil research showed some beneficial effects on the antioxidant status and LDL-C concentration after ovariectomy in rats [6]. Considering the chemical composition of these oils, it revealed that they contain analogous phytochemicals that the main active components include vitamin E, phytosterols and unsaturated fatty acids [7].

The characteristics of *M*oringa *oleifera* seed oil (MSO) can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids [8]. According to various studies, MSO is pleasant tasting, highly edible and resembles olive oil in its fatty acid composition [9]. On the other hand, the extract of MSO revealed various chemical constituents including α, γ, δ -tocopherols, sterol components [β-sitosterol, campesterol, stigmasterol and, Δ5-avenasterol, oleic acid, palmitic, stearic, behenic and arachidic acids] [10], which previous evidence indicated that these phytochemical associated with hypolipidemic activities [11]. A variety of mechanisms may account for the effects of dietary fats and oils on bone and lipid profiles, including alterations in calcium absorption, prostaglandin synthesis, formation of osteoblasts and lipid oxidation [12].

Previously, the leaf extract and other parts of *M. oleifera* have been reported to have hypocholesterolaemic action [13]. However, the certain efficacies of MSO have not yet been proven and no scientific report existing about the usefulness of MSO in improving lipid profiles, biochemical parameters and reproductive hormones. Therefore, the main purpose of this work

was to investigate the effects of the administration of MSO on lipid profiles, bone biomarkers and reproductive hormones in OVX rats.

2. Materials and Experiments

2.1 Chemicals and reagents

All of the reagents were analytical grades purchasing from Sigma Chemicals Company Co. (St. Louis, MO, U.S.A).

2.2 Experimental animals

A total of 50 of 3-month old female Wistar rats weighing 210-230 g were used. Bilateral ovariectomy and sham-operation were performed under sodium pentobarbital anesthesia during diestrus period to keep the consistent lowest levels of sex hormones. All animals were maintained in an environmentally controlled animal care with constant temperature (25 ± 0.5 ^{o}C), humidity (55 - 60%) and a 12 h light/ dark cycle. They were fed with a standard laboratory rat diet (CP Co., Ltd, Thailand), and supplied *ad libitum* drinking water.

Care and use of animals and the experimental protocols were approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology, Thailand.

2.3 Plant preparation and oil extraction

The mature seeds of moringa plant were collected from Lopburi Province, Thailand. Completed specimens were used for herbarium preparation. The genus and species of *M. oleifera* were confirmed by the botanists at the Royal Forest Department of Thailand and the specimens were deposited at the Royal Forest Department of Thailand for future references (BKF 186485). The total of 10 kg of the dried seeds were used for oil producing by cold press method [14]. The obtained oil was filtered and kept in bottles under refrigeration for further analysis.

2.4 Experimental procedures

The study was designed into two different series modified from Ferretti *et al.* [15], the first series was the preventive study and the second series was the recovery study. Each experimental series consisted of five sub-groups similarly; Group 1 (SHAM): Sham-operated controls received vehicle (1% Tween 80 in water); Group 2 (OVX): Ovariectomized controls received vehicle (1% Tween 80 in water); Group 3 (LDMSO): Ovariectomized rats treated with MSO 0.25 mL/100g BW/ day; Group 4 (HDMSO): Ovariectomized rats treated with MSO 0. 50 mL/100g BW/ day and Group 5 (E_2): Ovariectomized rats treated with 17β-estradiol 10 µg/kg BW/day. All animals were administered orally using gastric gavages feeding syringe. Treatments were performed at 09.00-12.00 AM and maintained in these conditions for 6 weeks. The administration process started on day 3 after ovariectomy for the preventive study and on day 60 after ovariectomy for the recovery study. Body weight of the animals was recorded weekly. At the end of the experiments, the animals were sacrificed under anesthesia and blood was collected from cardiac puncturing for serum analysis.

2.5 Urine and blood samples collection

To collect urine samples, rats were transferred to individual metabolic cages for a 12 h fasting period. During that time, the rats were free access to water. Urine was collected from 6.00 PM to 6.00 AM in acid-washed tubes and frozen at -20 °C until the requirement for analysis. At the end of the fasting period, the rats were anesthetized with CO_2 asphyxia. Blood collections for the two studies were performed by cardiac puncturing. All blood samples were collected in sample containers immediately and separated by centrifugation at 3,000 × *g* for 15 min (4 °C). Sera were aliquoted into small volumes and stored at -20 °C for biochemical determinations.

2.6 Determination for biochemistry parameters

Total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglyceride concentration in serum were measured using diagnostic kits (Raichem). The measurements of lipid profiles were carried out using Reflotron; Roch Diagnostics GmbH.

The levels of serum calcium (S-Ca), serum phosphorus (S-P) and serum alkaline phosphatase (S-ALP) activity were measured on an automatic analyzer (Ciba-Corning 550, USA) using a diagnostic reagent kit for biochemistry parameters determination. The urine calcium (U-Ca), urine phosphorus (U-P) and creatinine (Cr) concentrations were analyzed by the same method as the serum samples [16].

2.7 Statistical analysis

Data were expressed as mean ± S.E.M. ANOVA test with repeated measures was used to compare each parameter in each group after the treatment. Also, one-way ANOVA and Tukey's *post hoc* test were used to compare groups with each other. In addition, $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Effects of MSO on lipid profiles

Figure 1A-D shows TC and TG levels in preventive serum samples from the rats that were treated with MSO for 6 weeks. There was no significant difference in TC and TG levels between OVX control group and both the MSO treated groups. The HDL-C level in serum samples of HDMSO and E_2 treated rats was increased significantly ($p < 0.05$), while, the LDL-C level in these groups was decreased significantly ($p < 0.05$) when compared to control group. In recovery study, TC and TG levels in serum samples of E_2 and LDMSO treated rats were significantly decreased ($p < 0.05$) when compared to OVX control group. Additionally, the LDMSO and HDMSO treatment groups exhibited significant increases in the HDL-C and decreases in the LDL-C levels ($p < 0.05$) when compared to the OVX-control group (Figure 2A-D).

In the present study, MSO treatment restored LDL-C and HDL-C to control levels, and no significant differences between OVX treated with MSO and SHAM group, clearly indicated in

recovery study. The results from both series indicated that oral administration of MSO for 6 weeks in OVX rats produced/exhibited/possessed hypolipidemic effects and caused beneficial changes of total cholesterol, HDL-C and LDL-C levels. The mechanism(s) of underlying the hypolipidemic activity of MSO could be possibly explained by the presence of phytosterols that were consistent to previous reports [11, 18]. Meguro *et al.* [17] explained several mechanisms about the hypolipidemic activity of the plant sterols. It was reported that plant sterols which are structurally similar to cholesterol could displace cholesterol from mixed micelles, since they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol concentration and consequently lowers cholesterol absorption. Furthermore, dietary oils and fats compose of different types of fatty acids [FA]. It is possible that, MSO, which is rich in unsaturated fatty acids (UNSFAs) especially polyunsaturated fatty acids (PUSFAs) may exert the same effects [12]. PUSFAs stimulate the catabolic rate of LDL-C, thus resulting in the reduction of serum LDL-C [18]. Taken together, it is possible that MSO decrease the storage of cholesterol through those mechanisms mentioned above.

3.2 Effects of MSO on bone biomarkers in serum

The measured serum ALP, calcium and phosphorus levels in OVX rats that treated with MSO are shown in Table 1. Both OVX rats groups from preventive and recovery study showed significant elevated levels of serum ALP compared to SHAM operated control rats ($p < 0.05$). Whereas, OVX rats received E_2 of two series showed a significant decrease ($p < 0.05$) in ALP activity when compared to OVX control. The administration of all doses of MSO (LDMSO and HDMSO) decreased serum ALP significantly ($p < 0.05$).

After 6 weeks treatment, OVX groups showed significant ($p < 0.05$) changes in decreased serum calcium level when compared to SHAM operated control. The mean calcium concentration in serum was significantly ($p < 0.05$) higher in OVX rats received E_2 and HDMSO treated from preventive study and E_2, LDMSO and HDMSO from recovery study when compared to OVX control animals. Phosphorus concentration in OVX animals was higher ($p < 0.05$) when compared to SHAM groups. The serum phosphorus concentration was slightly high in OVX animals treated with MSO, but there were no significant differences in the serum phosphorus levels among LDMSO and HDMSO groups. A significant ($p < 0.05$) change in serum phosphorous level was observed in OVX rats administered with E_2.

These findings were similar to the results from dietary soybean, flaxseed and sesame oils which induced hypocholesterolemic and anti-osteoporotic effects in ovariectomized rats [19]. These vegetable oils can effectively improve ovariectomy-induced osteoporosis in rats. Therefore, they are considered as promising natural dietary supplements for the treatment of

postmenopausal osteoporosis in women. This was also consistent with many previous trials which reported that vegetable oils contain omega-3 and omega-6 polyunsaturated fatty acids which increased serum calcium and phosphorus concentrations and reduce urinary calcium and phosphorous excretion, thus enhanced bone formation [20-22].

Additionally, the biochemical parameters of bone formation (ALP) were also evaluated in this study. ALP is a non-collagenous protein secreted by osteoblast, which is essential for bone mineralization [23]. In OVX group, the ALP activities were increased compared to the SHAM control. Increases in ALP can probably be related to abnormal bone formation and stimulated osteoblastic activity with increased ALP concentration in the serum [24]. The administration of MSO improves these parameters, and might reduce bone reabsorption and increase the bone formation [21]. The beneficial effect of MSO on the reduction of ALP activity might be due to the presence of high calcium concentration in the oil [25]. The raised bone ALP activity occurring with ovariectomy could contribute to high bone turnover rate, being characterized by an increase in both bone reabsorption and formation [26]. The positive role of medical herbs supplemented diets was also achieved by the observed improvement of bone metabolic markers, ALP and acid phosphatase (ACP) [27] Phytoestrogenic herbs exhibited comparable effects regarding all tested parameters, indicating almost similar ability of herbs to protect against bone loss [21].

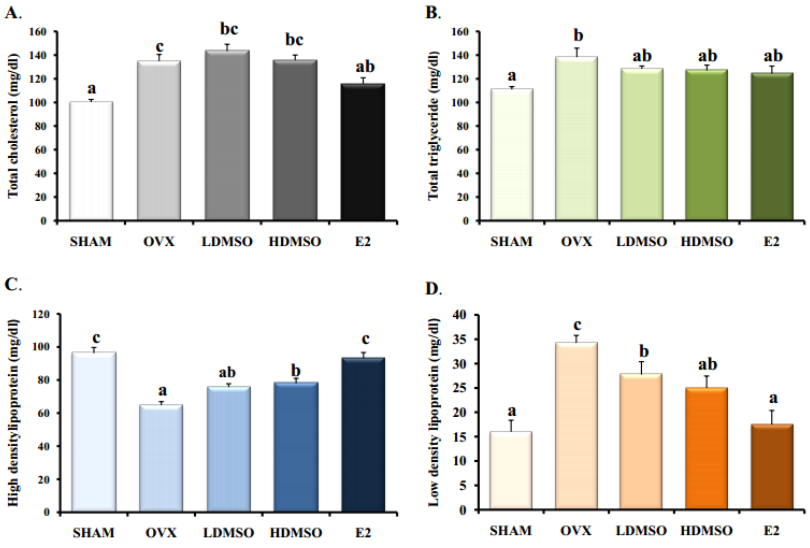

Figure 1 Effects of MSO on lipid profiles of preventive study. The total cholesterol level (A), triglyceride level (B), high density lipoprotein level; HDL-C (C) and low density lipoprotein level; LDL-C (D). All treatments are shown; SHAM received vehicle, OVX received vehicle, OVX received 0.25 mL/100g BW MSO (LDMSO), OVX received 0.50 mL/100g BW MSO (HDMSO) and OVX received E_2, respectively. Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. Values that do not share the same superscript letters are significantly different ($p < 0.05$).

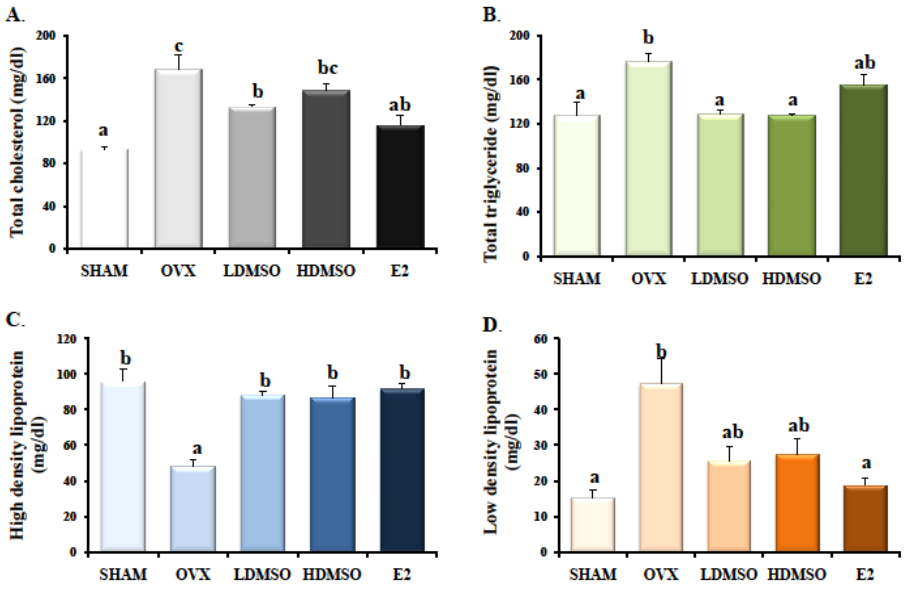

Figure 2 Effects of MSO on lipid profiles of recovery study. The total cholesterol level (A), triglyceride level (B), high density lipoprotein level; HDL-C (C) and low density lipoprotein level; LDL-C (D). All treatments are shown; SHAM received vehicle, OVX received vehicle, OVX received 0.25 mL/100g BW MSO (LDMSO), OVX received 0.50 mL/100g BW MSO (HDMSO) and OVX received E_2, respectively for 4 weeks. Values are expressed as mean ± S.E.M. Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. Values that do not share the same superscript letters are significantly different ($p < 0.05$).

Table 1 Effects of MSO on serum-bone biomarkers of ovariectomized rats treated for 6 weeks.

Groups	Treatments	Serum alkaline phosphatase (IU/L)	Serum calcium (mg/dL)	Serum Inorganic phosphorus (mg/dL)
Preventive	SHAM	32.33 ± 11.40^{d}	10.97 ± 0.79^{a}	9.78 ± 0.49^{d}
	OVX	72.33 ± 10.34^{a}	9.15 ± 0.20^{c}	12.20 ± 0.91^{a}
	LDMSO	50.67 ± 8.09^{bc}	9.67 ± 0.16^{bc}	11.75 ± 0.71^{b}
	HDMSO	65.00 ± 10.58^{b}	10.33 ± 0.70^{b}	11.55 ± 0.79^{b}
	E_2	40.67 ± 5.81^{c}	10.42 ± 0.38^{b}	10.89 ± 0.39^{c}
Recovery	SHAM	56.00 ± 5.88^{d}	10.45 ± 0.26^{c}	11.93 ± 0.33^{c}
	OVX	96.00 ± 8.32^{a}	11.50 ± 0.45^{a}	13.62 ± 1.64^{a}
	LDMSO	80.67 ± 9.82^{b}	11.28 ± 0.22^{a}	13.32 ± 1.39^{a}
	HDMSO	94.66 ± 5.33^{b}	10.98 ± 0.22^{b}	11.74 ± 0.92^{c}
	E_2	76.50 ± 4.03^{c}	10.03 ± 0.41^{c}	12.66 ± 1.08^{b}

Values are expressed in mean ± S.E.M. of five animals. Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. Means with different superscripted letters in the same column indicate statistical significance ($p < 0.05$).

Table 2 Effects of MSO on urine-bone biomarkers of ovariectomized rats treated for 6 weeks.

Groups	Treatment groups	Urinary Calcium (mg/dL)	Urinary phosphorus (mg/dL)	Urinary creatinine (μmol/L)
Preventive	SHAM	8.12 ± 0.08^{c}	12.86 ± 2.10^{d}	117.05 ± 1.80^{c}
	OVX	11.57 ± 0.02^{a}	33.37 ± 7.17^{a}	154.83 ± 8.83^{a}
	LDMSO	10.35 ± 0.01^{b}	23.03 ± 3.35^{b}	111.00 ± 5.63^{bc}
	HDMSO	10.10 ± 0.05^{b}	18.76 ± 2.86^{c}	124.23 ± 7.67^{b}
	E_2	9.37 ± 0.01^{b}	15.72 ± 3.13^{d}	117.63 ± 15.13^{c}
Recovery	SHAM	13.07 ± 2.53^{d}	21.80 ± 1.01^{c}	129.40 ± 10.39^{c}
	OVX	28.30 ± 1.05^{a}	33.96 ± 1.48^{a}	168.83 ± 8.65^{a}
	LDMSO	24.60 ± 1.76^{b}	28.13 ± 2.67^{b}	131.00 ± 12.14^{b}
	HDMSO	24.76 ± 1.50^{b}	29.27 ± 2.54^{b}	124.67 ± 11.88^{c}
	E_2	19.05 ± 1.11^{c}	27.16 ± 0.99^{b}	115.17 ± 8.78^{d}

Values are expressed in mean ± S.E.M of five animals. Data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test. Means with different superscripted letters in the same column indicate statistical significance ($p < 0.05$).

3.3 Effects of MSO on bone biomarkers in urine

Table 2 shows the level of urine calcium, phosphorus and creatinine. The analysis of urine samples of OVX control rats revealed significant ($p < 0.05$) increases in calcium, phosphorous and creatinine concentrations when compared to SHAM control rats. In OVX rats fed with E_2, all parameters were decreased to normal values and similar to the samples from SHAM group.

Urine calcium was slightly reduced as compared to OVX control group. In preventive study, there was a slightly alteration in urine calcium in MSO treated animals, and did not reach a statistical significance. On the other hand, the recovery study showed significant ($p < 0.05$) increases in urine calcium level compared with OVX group.

Further investigations regarding blood and urine biochemical parameters were also recorded in OVX rats. It is well known that calcium and phosphorus are widely accepted as phenotype markers for bone formation [28]. The present work also indicated that the levels of serum and urinary Ca and P in OVX rats were significantly higher than the control group. These results are in agreement with the levels of serum Ca and P were increased in the ovariectomized rats [29]. In the present study feeding MSO to OVX rats significantly restored the decreased serum and urinary calcium and phosphorus levels induced by ovariectomy to normal levels, while it decreased urine calcium and phosphorous concentrations. These results suggested that oral supplementation with MSO were effective in inhibiting bone reabsorption and in increasing bone formation [30].

Furthermore, urine creatinine levels from all treatments of MSO treated rats in both studies were significantly decreased ($p < 0.05$) when compared to OVX control group. The levels of creatinine in urine may point to a kidney disease, certain muscular and neuromuscular disorders, or a blockage in the urinary tract. The MSO administration in OVX rats for six weeks exhibits the potential improve urinary creatinine level. Further work, nephrotoxicity study on long-term consumption of oil extract is needed to evaluate. The mechanism(s) by which oil supplementation can modulate the bone tissues should be investigated as well.

4. Conclusions

These results demonstrated that MSO has multiple metabolic benefits in OVX rats. The oral administration of MSO tended to decrease LDL-C levels but increase HDL-C levels and to restore the serum levels of Ca, P and ALP. This oil also decreased urinary Ca, P and creatinine. Taken together, MSO could be protective effects against cardiovascular disease and anti-osteoporosis after menopause.

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