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Effect of Moringa Leaves and Seeds on Osteoporosis in Rats

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ABSTRACT

Osteoporosis (OP) speaks to most regular metabolic bone maladies. This study was conducted to study the impact of *Moringa oleifera* leaves and seeds and their blends on osteoporosis in rats. Thirty five adult female albino of rats, of (180 ± 10 g) were divided into five groups, the first group, negative control group (-ve) and fed on basal diet only, the other four groups (7 rats each) were fed on basal diet containing 100 mg Prednisone Acetate as source of glucocorticoid/ kg diet to induce osteoporosis for two weeks. One group of them was served as a positive control group, the other three groups were fed on prednisone acetate diets containing dried moringa leaves (2.5%), moringa seeds (2.5%) and 2.5% of their combination at (1:1), respectively. Blood samples and femur bones were collected for estimating both serum and bone markers of osteoporosis. The results indicated that, supplementation with *Moringa oleifera* leaves and seeds and their combinations significantly increased ($P<0.05$) serum Ca and P in osteoporotic rats. There was significant increase ($P<0.05$) in serum free thyroxin (T4) and a significant decrease ($P<0.05$) in parathormone (PTH) in osteoporotic rats. Femur bone mineral density (BMD) were also significantly increased. The present examination suggested utilizing *Moringa oleifera* leaves and seeds and their blends as a good anti-osteoporotic activity.

Keywords: Osteoporosis, Moringa oleifera, leaves, seeds, flavonoids, rats.

INTRODUCTION

Osteoporosis is a multifactorial illness affected by hereditary and different natural variables (Riggs and Melton, 1992 and Kanis *et al*., 1994). Gerdhem, (2013) revealed that osteoporosis prompts diminish in a bone mass and microarchitectural retrogradation of the tissue, prompting skeletal delicacy and potential outcomes of break, intensifying the life of the patient. Osteoporosis emerges because of loss of bone honesty.

To overcome the extensive variety of symptoms created by the engineered drugs, there is expanding requests for 'green pharmaceutical' a more advantageous and more secure choice with less or no reactions (Shirwaikar *et al*., 2010). Remedial impacts of plant medications are accounted for to be because of aggregate activity of various constituents through multipathways pointing multitargets (Jia *et al*., 2012).

Osteoporosis is related with irritation and expanded oxidative pressure and plant based drugs that objective these related illnesses alongside the sickness, are relied upon to bring attractive impacts. Oxidative pressure has been accounted for to lessen separation and survival of osteoblasts (Basu *et al*., 2001 and Rao and Rao, 2007) what's more, antioxidants from plants and organic products are known to diminish the harming impacts of receptive oxygen species (ROS) (Sharan *et al*., 2009). The auxiliary metabolites are accounted for to increment osteoblastic movement by expanding the outflow of BMP-2, BMP-4, Runx 2 and cyclin D1.The secondary metabolites are reported to increase osteoblastic activity by increasing the expression of BMP-2, BMP-4, Runx 2 and cyclin D1. Some plant mixes likewise restrain the declaration of IL-6, TNFα, COX-2 and PGE2, vital for the separation of osteoclasts (Jia *et al*., 2012).

Moringa oleifera Lam. has a place with the family *Moringaceae* (sort Moringa) (Leone *et al*., 2015a). It is an extraordinarily nutritious vegetable tree with an assortment of potential employments. The moringa can possibly enhance nourishment, help sustenance security, encourage country improvement, and bolster maintainable landcare. It is viewed as one of the world's most helpful trees, as relatively every part can be utilized for sustenance or has some other gainful property. It is one of the most extravagant plant wellsprings of Vitamins A, B, C, D, E and K. The crucial minerals present in Moringa incorporate Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc (Mensah *et al*., 2012).

All parts of the Moringa tree are appropriate for human and creature utilization. The leaves, which are wealthy in protein, minerals, β-carotene and cancer prevention agent mixes, are utilized for human and animal nutrition but also in traditional medicine (Leone *et al*., 2015 a,b). Moringa powder contains sufficient proportion of vitamins, minerals, protein, phenols and distinctive phytonutrients. This makes the tree a pharmaceutical for an extensive variety of disorders (Gedefaw, 2015).

The leaves are the most nutritious part of the plant, being a significant source of vitamin B6, vitamin c, β-carotene, magnesium and protein (Chandramouli *et al*., 2012). The seeds are also known to exert its protective effect by decreasing liver lipid peroxides and, as an antimicrobial agent (Faizi *et al*., 1998). The base of *Moringa oleifera* were appeared to have antihelmithic, rubefacient, carminative, antifertility, mitigating, stimulant in disabled torments; go about as a cardiovascular / circulatory tonic, utilized as a purgative, abortifacient, in treatment of stiffness, aggravations, articular torments, bring down back or kidney torment and obstruction (Anwar *et al*., 2005).

Various researchers have assigned that this plant is having various phytochemicals, especially phytoestrogens which can have positive effect on bone (Burali *et al*., 2010). Zhang *et al*., (2008) illustrated that flavonoids can aggravate osteoblastic proliferation and differentiation, (Vali *et al*., 2007) proved that Epigallocatechin-3-gallate such as flavonoids induces bone mineralization and bone nodule formation.

Aim of the study

As *Moringa oleifera* leaves and seeds are wealthy in different flavonoids and phytoestrogens, accordingly, this work was intended to investigate the potential impacts of *Moringa oleifera* leaves and seeds and their blends on osteoporosis in rats.

MATERIALS AND METHODS

Materials

Moringa leaves: *Moringa oleifera* leaves were collected from the plantations in Egypt. Seed of *Moringa oleifera* were obtained from a Local market. Moringa leaves and seeds were identified and authenticated by a plant taxonomist, Faculty of agriculture, Ain Shames University.

Rats: Thirty five adult female albino of Sprague Dawley strain rats (3 months old) were obtained from Helwan Farm, Ministry of Health and Population, Cairo, Egypt.

Diet: Casein, vitamins, cellulose, minerals and methionine were obtained from Morgan Company for Chemicals, Cairo, Egypt.

Chemicals: Kits for biochemical analysis were purchased from Biodiagnostic Company for Pharmaceutical and chemicals, Dokki, Egypt. Prednisone acetate as source of glucocorticoid (GC) was obtained from Morgan Chemical Factory, Cairo, Egypt.

Methods

Preparation of dried Moringa leaves: The leaves of the plant were cleaned thoroughly. A part of the fresh leaves appropriated has been dried at Solar Energy Department, National Research Institute, then minced to powder by milling using a locally Milling machine and then kept in plastic sachets at room temperature (25˚C ± 2˚C) until use.

Analytical Methods of Moringa leaves and seeds:

Moisture, Protein, fat, fiber and ash were determined in Moringa leaves and seeds according to the method recommended by A.O.A.C. (2000). Carbohydrates are calculated according to the equation (total carbohydrates= (Moisture+ fats +protein + ash) -100

Calcium was determined in the diluted solution of ash samples by using emission flam photometer. The other minerals (iron, phosphorus and magnesium) were determined by Atomic absorption spectrophotometer (Nzikou *et al*., 2009).

Experimental Animal Design:

Thirty five adult female albino of rats, of (180 ± 10 g) were placed in well aerated cages under hygienic condition and fed for one week on basal diet for adaptation, then were divided into five groups as follows:

The first group (n=7 rats) was kept as negative control group (-ve) and fed on basal diet only according to Reeves *et al*., (1993). The other four groups (7 rats each) were fed on basal diet containing 100 mg Prednisone Acetate as source of glucocorticoid/ kg diet to induce osteoporosis for two weeks (Liao *et al*., 2003). One group of them was served as a positive control group, the other three groups were fed on prednisone acetate diets containing dried moringa leaves (2.5%), moringa seeds (2.5%) and 2.5% of their combination at (1:1), respectively.

At the end of the study (8weeks) the rats were fasted for 12 hour, and then sacrificed under ether anesthesia. Blood samples were obtained from medial canthus of the eyes of rats by means of fine capillary glass tubes in a centrifuge tube without any anticoagulant and centrifuged for 20 minutes at 3000 r.p.m. to obtain serum.

Chemical analysis:

Serum levels of calcium and phosphorus were determined according to Gindler and King, (1972) and El-Merzabani *et al*., (1977), respectively. Additionally, serum PTH was estimated through radioim munoassay (RIA) according to Deftos, (1990). Free thyroxine was determined in serum according to the method described by kit for rat (Enzyme-linked immuneosorbent assay Kit). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to (Reitman and Frankel, 1957).

Measurement of bone mineral density: The BMD of the left femur bone was estimated in anesthetized rats using dual energy X-ray absorptiometry (Gao *et al*., 2013).

Statistical analysis.

Results were expressed as mean±standard Error (SE). Data were analyzed statistically by SPSS program, one way ANOVA followed by post hoc multiple were used to make a comparison among different groups (Snedecor and Cochron, 1989).

RESULTS

The Nutritive value of *Moringa oleifera* leaves, seeds in Table (1) indicated that, *Moringa oleifera* leaves, seeds contain high amount of moisture, carbohydrates and fiber, and low amount of proteins and fats. Regarding the minerals content, it is cleared that, *Moringa oleifera* leaves, seeds contain a high amount of Ca, P, Fe and Mg.

Table 1. Nutritive value of Moringa leaves and seeds

Nutrients		Leaves	Seeds
Nutrients (g/100 g)	Moisture	73.0	47.5
	Proteins	5.3	16.3
	Fats	1.7	21.2
	Carb.	13.0	6.8
	Fiber	0.8	2.58
	Ash	7.0	8.2
Minerals (mg/100 g)	Ca	390	242
	P	200	186
	Iron	18.4	7.9
	Magnesium	250	410

Results illustrated in Table (2) revealed the effect of Moringa (leaves, seeds and their mixture) on serum contents of calcium (Ca) and phosphorus (P) on osteoporotic rats. Serum calcium and phosphorus of the positive control group were significantly ($P<0.05$) decreased, compared to the negative control rats. Feeding rats on Moringa leaves, seeds or mixture of Moringa (leaves and seeds) caused a significant increase ($P<0.05$) in serum calcium and phosphorus as compared to positive control group. No significant changes in serum levels of Ca and P was observed between the groups fed on either Moringa leaves or seeds. It was observed that the group fed on a mixture of Moringa (leaves and seeds) had significant increase in serum Ca and P contents as compared to the other tested groups. Moreover, Serum P level at the mixture group had no significant difference as compared to the negative control group.

Table (3) showed the effect of Moringa (leaves, seeds and their mixture) on serum T4 and PTH on osteoporosis in rats. The positive control rats had significant ($P<0.05$) decrease in serum level of T4 and an increase in serum PTH as compared to the negative control group. Supplementation with Moringa (leaves, seeds or their mixture) significantly increased ($P<0.05$) the lowered levels of serum T4 but significantly decreased ($P<0.05$) the

elevated levels of serum PTH, respectively as compared to the positive control rats. Moreover, there was no significant difference in serum levels of T4 and PTH among the three tested groups.

Table 2. Effect of Moringa leaves, seeds and their mixture on serum calcium and phosphorus contents in osteoporotic Rats

Parameters	Ca	P
Groups	(mg/dl)	
Control (-ve)	13.85±0. 15[a]	7.10±0.30[a]
Control (+ve)	7.95±0. 45[d]	3.40±0.30[c]
Moringa leaves (2.5%)	10.10±0.20[c]	4.95±0.05[b]
Moringa seeds (2.5%)	10.35±0.65[c]	5.20±0.60[b]
Mix of Moringa (Leaves and seeds (2.5%)	11.80±0.20[b]	6.55±0.15[a]

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Table 3. Effect of Moringa leaves, seeds and their mixture on serum thyroxin and parathyroid hormone in osteoporotic rats

Parameters	T4	PTH
Groups	(µg/dl)	(pg/mL)
Control (-ve)	8.30±0.20[a]	0.85±0.15[c]
Control (+ve)	3.40±0.30[c]	3.15±0.15[a]
Moringa leaves (2.5%)	5.60±0.40[b]	1.85±0.15[b]
Moringa seeds (2.5%)	6.05±0.55[b]	1.65±0.15[b]
Mix of Moringa (Leaves and seeds (2.5%)	6.80±0.30[ab]	1.45±0.15[b]

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

Effect of diet supplemented with Moringa leaves, seeds and their mixture on Bone Mineral Density of osteoporotic rats was shown in Table (4). The mean bone mineral density of the positive control group was decreased (P<0.05) significantly, compared to the negative control rats. The supplementation with Moringa leaves, seeds and their mixture significantly (P<0.05) increased the mean value of bone mineral density, compared to the positive control group. There was no significant change in BMD between the groups given Moringa leaves or seeds. However there was a significant increase (P<0.05) in BMD for rats fed a basal diet and supplemented with a mixture of Moringa seeds and leaves as compared to the other treated groups.

Table 4. Effect of Moringa leaves, seeds and their mixture on Bone Mineral Density in femur bone of osteoporotic rats.

Parameters	Bone Mineral Density
Groups	(g/cm^3)
Control (-Ve)	0.09±0.10 [c]
Control (+Ve)	0.06±0.11 [d]
Moringa leaves (2.5%)	0.11±0.51 [b]
Moringa seeds (2.5%)	0.11±0.44 [b]
Mix Moringa (Leaves and seeds (2.5%)	0.14±0.13 [a]

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

The data in Table (5) showed the effect of Moringa (leaves, seeds and their mixture) on serum liver functions of osteoporotic rats. The activities of liver functions were significantly increased (P<0.05) in the positive control group, compared with the corresponding value of normal control group as a result of feeding Prednisone Acetate diet. Supplementation with Moringa leaves, seeds or their mixture at the tested level significantly decreased (P<0.05) the higher levels of both serum ALT and AST compared to the positive control group. Moreover, there was no significant change in serum ALT or AST among the three treated groups. It was clear there was no significant change in serum ALT among the group fed on mixture of Moringa (leaves and seeds) and the negative control group. The best results of liver functions were recorded at the group fed on basal diet supplemented with 2.5% of Moringa (leaves and seeds) as a mixture.

Table 5. Effect of Moringa leaves, seeds and their mixture on serum liver functions in osteoporotic rats

Parameters	AST	ALT
Groups	(µ/L)	
Control (-ve)	92.30±2.30[d]	35.25±2.25[c]
Control (+ve)	121.50±2.50[a]	55.20±1.20[a]
Moringa leaves (2.5%)	112.80±2.50[b]	43.50±2.50[b]
Moringa seeds (2.5%)	109.50±0.50[bc]	45.30±1.30[b]
Mix Moringa (Leaves and seeds) (2.5%)	104.00±2.00[c]	41.15±1.15[bc]

Values are expressed as means ± SE.
Values at the same column with different letters are significantly different at P<0.05.

DISCUSSION

The real bone malady is osteoporosis, a fundamental skeletal illness, with an ensuing increment in bone delicacy and defenselessness to break. Menopause or estrogen inadequacy is the fundamental driver of osteoporosis (Shuid *et al*., 2011). The expanding proof of osteoporosis and its related cracks have turned out to be worldwide medical problems (Potu *et al*., 2009). Be that as it may, hormone substitution treatment has been turned out to be adequate in avoiding bone misfortune, yet, it isn't attractive to numerous ladies because of its side effects (Das, 2002).

Mbikay, (2012) announced that the utilization of normal items as an elective treatment in mending and treatment of different ailments has been on the ascent over the most recent couple of years. *Moringa oleifera* leaves, seeds, bark, sap, roots and blossoms are broadly utilized in customary solution, and the leaves and juvenile seed cases are utilized as nourishment items in human sustenance. Therefore, this work was intended to investigate the potential impacts of *Moringa oleifera* leaves and seeds and their blends on osteoporosis in rats.

Presence of different sorts of antioxidant compounds mixes make *Moringa oleifera* leaves a significant wellspring of regular cell reinforcements (Anwar *et al*., 2007) and a good source of nutraceuticals and functional components as well (Makkar and Becker, 1996). The Moringa plant has been devoured by people during the time in various culinary ways (Iqbal and Bhanger, 2006). All parts of the plant are utilized for its healthful esteem, indicated restorative properties and for taste and flavor as a vegetable and seed. *Moringa leave* is a safe natural antioxidant and is found as a potential hotspot for phenolics mixes, vitamin A, vitamin C, and E (Perumal and Klaus, 2003 and Yang *et al*., 2006).

The concoction structure of *Moringa oleifera* leaves, seeds in Table (1) uncovered a high substance of dampness, starches and fiber, yet low in proteins and fats.

Concerning minerals content, it is cleared that, contains an abnormal state of Ca, P, Fe and Mg. The healthful examinations uncovered the nearness of carbohydrates, ascorbic acid; fibre, protein as well as iron, calcium vitamin C, potassium, magnesium and vitamin A (Doss *et al*., 2009). These results were also in accordance with some studies (Moyo *et al*., 2011; Oluduro, 2012 and Nweze and Nwafor, 2014). Fozia *et al*., (2012) say that *Moringa* powder contains adequate measure of vitamins, minerals, protein, phenols and different phytonutrients. This makes the tree a solution for various illnesses.

Gedefaw, 2015; Gopalakrishnan *et al*., 2016 and Kane *et al*., (2017) found that *Moringa*, is one of the most extravagant plant wellsprings of Vitamins and minerals, for example, Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. These outcomes were coordinating with the present study.

Consequences of the present study demonstrated that sustaining rats on *Moringa* leave*s*, seeds or blend caused a noteworthy increment in the level of serum Calcium and Phosphorus. Calcium, phosphate, and vitamin D are basic for sound bone structure and function (Sophocleous *et al*., 2003).

The abatement in serum levels of calcium and phosphorus in rats experiencing osteoporosis actuated as detailed in this examination was like the past reports (Tamir *et al*., 2001). The diminished serum calcium levels were caused because of estrogen inadequacy in rats experiencing osteoporosis initiated (Choi and Seo, 2013). Experimental diet supplemented with Moringa leaves or Moringa seeds significantly increased ($P<0.05$) the mean values of these biochemical markers and this could be due to an increased osteoblastic activity, consequently enhancing bone formation (Srikanta *et al*., 2011).

Ilich and Kerstetter, (2000) showed that nutrition has a critical job in upkeep of good bone. Particular hazard factors incorporate low dietary calcium and additionally phosphorus, magnesium, zinc, press, fluoride, copper, vitamins A, K, E,D and C. Flood sodium is likewise a hazard factor.

With respect to metabolic hormones, parathyroid hormone, protein hormone discharged by the parathyroid organ, is a noteworthy controller of bone digestion and calcium homeostasis (Papavasiliou *et al*., 2003). The present study showed that rats experiencing osteoporosis brought about an exceptionally huge increment in PTH levels contrasted with the control gathering. The acquired results are in accordance with Taguchi *et al*., (2006).

The present results denoted that supplementation with Moringa (leaves, seeds or their mixture) significantly increased the lowered levels of serum T4 and significantly decreased the elevated levels of serum PTH. These results were in accordance with the finding reported by Norazlina *et al*., (2010). Tahiliani *et al*., (2000) reported that *Moringa* leaves go about as a thyroid hormone controller. Seeds of *moringa* help in treating joint inflammation, stiffness, gout, cramp and anti-inflammatory agents (Rockwood *et al*., 2013).

Bone Mineral Density significantly ($P<0.05$) increased in groups fed basal diet and supplemented with Moringa leaves, seeds or their mixture. The minerals found in *M. oleifera* play both curative and preventive role in combating human disease. For instance, Ca is a multifunctional supplement fundamental to the body digestion (Sizer and Whitney, 1999), but Ca insufficiency prompts osteoporosis. In this way, *M. oleifera* is viewed as a characteristic solution for osteoporosis (Howard, 2014). The reproducer of *moringa* blooms decreased the levels of rheumatoid factor, TNF-alpha and IL-1 in joint rats, in this way, *moringa* can be a strong treatment for joint inflammation (Mahajan and Mehta, 2009).

The real serum hepatic enzymes are AST and ALT and utilized for liver function test. The higher exercises of these compounds in serum are a sign of liver damage (Islam *et al*., 2011). The present outcomes uncovered that supplementation with *Moringa* leaves, seeds or their blend at the tried level significantly decreased ($P<0.05$) the raised levels of both serum ALT and AST contrasted with the positive control group. These results were in accordance with Sheikh *et al*., (2014) who reported that the addition with *Moringa* leaves significantly reduced arsenic-actuated height of liver activities. Sadek *et al*., (2015) reported that the concentrate of *Moringa* leaves treated groups demonstrated a significant reduction in the levels of liver enzymes, when contrasted with the diabetic untreated control. These discoveries are as per the outcomes on these biochemical parameters (Usha *et al*., 2008 and Atawodi *et al*., 2010).

Siddiq *et al*., (2005) and Sreelatha and Padma, (2009) reported that methanolic concentrate of *M. oleifera* leaves had a high antioxidant activity, which might be credited to the closeness of polyphenolics and other antioxidant substances. Nascimento *et al*., (2013) and Coppin *et al*., (2013) suggest that *M. oleifera* might be an essential source of natural antioxidants. Mónica *et al*., (2015) detailed that *M. oleifera* contain a high centralization of vitality, supplements, minerals, and phenolic constituents, which represent a good source of natural antioxidants. In this manner, the helpful capability of *M. oleifera* might be because of the nearness of these constituents.

CONCLUSION

The present study recommended using *Moringa oleifera* leaves and seeds and their combinations as a good anti-osteoporotic activity.

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تأثير أوراق وبذور المورينجا علي هشاشة العظام في الفئران

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ترقق العظام يمثل معظم أمراض العظام الأيضية شيوعا. أجريت هذه الدراسة لمعرفة تأثير أوراق وبذور نبات المورينجا وخليطهما على هشاشة العظام في الفئران. تم تقسيم عدد ٣٥ من اناث الفئران البالغة من سلالة ألبينو (١٨٠ ± ١٠ جم) إلى خمس مجموعات ، المجموعة الأولى وهي المجموعة الضابطة السالبة وتم تغذيتها على النظام الغذائي الأساسي فقط. وتم تغذية المجموعات الأربعة الأخرى (٧فئران لكل منهما) على النظام الغذائي الأساسي المحتوي على ١٠٠ ملجم بريدنيزون أسيتات كمصدر للكورتيزون/ كجم من النظام الغذائي لاحداث هشاشة العظام لمدة أسبوعين. تم اختيار مجموعة واحدة منهم كمجموعة ضابطة موجبة ، وكانت المجموعات الثلاثة الأخرى تتغذى على وجبات بريدنيزون أسيتات تحتوي على أوراق المورينجا المجففة (٢.٥ ٪) وبذور المورينجا (٢.٥ ٪) و ٢.٥ ٪ من خليطهما بنسبة (١: ١) ، تم جمع عينات الدم وعظام الفخذ لتقدير نسبة الهشاشة فى الدم والعظم. أشارت النتائج إلى أن الوجبات الغذائية المدعمة بأوراق نبات المورينجا والبذور وخليطهما أدت الي حدوث ارتفاع معنوي ($P<0.05$) في مستوى الكالسيوم والفسفور. كانت هناك زيادات معنوية ($P<0.05$) في هرمون الثيروكسين في الدم (T4) وانخفاض هرمون الباراثيروويد (PTH). كما تم زيادة محتوى عظم الفخذ (BMC) من الاملاح المعدنية. أوصت الدراسة الحالية باستخدام أوراق وبذور المورينجا وخليطهما لما لها من نشاط جيد لمكافحة هشاشة العظام.