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The Role of Avocado, Flaxseed Oils and Their Synergistic Effects on Osteoporotic Female Rats

Hadeer H. Mohammed, Nehad Naem Hamed Shosha* and Fatma Abd El-Hamed Khalil

Biochemistry and Nutrition Department, Women's College, Ain Shams University, Cairo, Egypt

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***Corresponding author:** Nehad Naem Hamed Shosha

Abstract **Original Research Article**

Osteoporosis is a skeletal disorder characterized by reduction of bone mineral density (BMD) and bone strength leading to increased risk of fractures. Many secondary factors may contribute to the bone fragility or osteoporosis. The current study evaluates the effect of avocado and flaxseed oils and their synergetic effect on the secondary osteoporosis induced by glucocorticoid treatment. For this purpose, this study conducted on Sixty female albino rats were divided into 2 main groups; healthy and osteoporotic groups. Osteoporotic rats were treated with 6mg prednisolone/kg body weight daily for three weeks, then subdivided into five subgroups, each subgroup consisted of 10 rats as follow; 10 rats served as osteoporotic rats, 10 rats served as osteoporotic rats fed on Calcium (Ca) supplement (7g calcium carbonate/ kg diet). 10 rats of osteoporotic rats fed on normal diet containing 4% avocado oil with Ca supplement, 10 rats of osteoporotic rats fed on normal diet containing 4% flaxseed oil with Ca supplement. Finally, 10 rats of osteoporotic rats fed on normal diet containing 4% both oils mixture with Ca supplement. The obtained data revealed that, glucocorticoid treatment caused significant reduction in BMD, bone mineral content (BMC), bone formation markers as serum osteocalcin (OC), alkaline phosphatase (ALP) and estradiol (E_2) levels and significant increase in bone resorption markers as acid phosphatase (ACP) and parathyroid hormone (PTH) levels. Also, significant increase in Ca and phosphorus (P) bone resorption and urine excretion indicating the onset of osteoporosis. Whereas, feeding of osteoporotic rats by Ca supplement with avocado or/and flaxseed oils caused significant elevation in BMD and BMC indicated by significant improvement in the levels of serum, urine and bone Ca and P levels and bone formation markers with significant reduction in bone resorption markers. In conclusion, using oils mixture with Ca supplement induced the highest improvement of osteoporosis.

Keywords: Osteoporosis, Avocado, Flaxseed, Oils, bone, Rats.

INTRODUCTION

Osteoporosis represents an imbalance between bone formation and bone resorption, results in a reduction in bone mass, bone integrity and an increase in the incidence of fractures [1]. The most common risk factors for osteoporosis are age and female sex. Women experience more hip fracture than men this is thought to be related to the effect of menopause [2], more than two million osteoporotic fractures occur annually in the US [3]. The fractures caused by osteoporosis have a great impact on public health, since they are associated with increased morbidity, mortality, long hospital stays, poor quality of life and high economic cost [4].

Corticosteroid (GC) - induced osteoporosis is the most common cause of secondary osteoporosis especially in young people [5]. Bone loss and fracture risk increase rapidly after the initiation of corticosteroid therapy and are proportional to dose and treatment duration. Prevention should be considered in all patients beginning corticosteroid therapy, especially as the underlying inflammation therapy impairs bone quality [6]. The global prevalence of fractures in patients receiving long-term GC has been reported to be 30–50% [5].

Calcium is an essential element in bone mineralization and formation being the key component of hydroxyapatite (bone mineral complex) and its use as a monotherapy for osteoporosis were previously reviewed [7]. Treatment of osteoporosis requires supplementation with calcium and vitamin D, particularly if dietary intake is in adequate. Most patients require calcium supplementation to meet the recommended daily intake of 1000 to 1500 mg/d [8].

Nutrition is considered one of main modifiable factors that affects bone health. Many nutritional and non-nutritional factors have attracted the interest in research on their effect on osteoporosis treatment. The n-3 polyunsaturated fatty acids (PUFAs) play important roles in the regulation of a variety of biological processes including bone metabolism [9] by Increasing BMC, BMD and inhibit bone resorption [10] by various mechanisms including inhibition of receptor activated nuclear factor κβ ligand (RANKL)- induced osteoclastogenesis and osteoclast differentiation [11]**.** Moreover, there is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. Phenolic compounds exhibit a considerable free-radical scavenging activity, which is determined by their reactivity as hydrogen or electron donating agents, the stability of the resulting antioxidant derived radical, their reactivity with other antioxidants and, finally, their metal chelating properties [7].

Persea americana, Mill., also known as avocado, is a tropical plant native to Central America containing a high amount of lipids and essential minerals like magnesium, potassium and phosphorus in the mesocarp [12]. It belongs to the *Lauraceae* family. It has been traditionally cultivated for food and medicinal purposes due to its high nutritive content and therapeutic properties with several medicinal effects, which includes hypotensive, hypoglycaemic, anti-viral, analgesic and anti-inflammatory [13]. This fruit is a rich potential source of oil (15–30 g/100 g of fruit), mostly monounsaturated and a good source of linoleic acid [14]. It also contains high levels of antioxidants including polyphenols, tocopherols, and carotenoids which have shown positive health benefits [15]. Studies in human and animal models have shown that avocado oil helps to control weight, reduces the risk of diabetes [16], and normalizes blood lipid profile [17], Other studies reported the presence of functional molecules such as glutathione [18], a molecule related to decreased risk of cancer. On the other hand, the unsaponifiable components, rich in antioxidant molecules [19], have also shown beneficial effects on anti-inflammatory processes related to the development of cancer [20]. In addition to, the phytochemical components of avocado oil that related to the disease manifestations associated with an altered metabolic profile [15]; so overall, it is expected that all the beneficial properties of avocado oil will achieve positive bone health effects.

Flaxseeds have nutritional characteristics and are rich source of n-3 fatty acid: α-linolenic acid (ALA), short chain PUFAs, phytoestrogenic lignans (secoisolariciresinol diglycoside), and an array of antioxidants [21]. Dietary phytoestrogens are compounds that are estrogen like in structure and can elicit both weak estrogenic and antiestrogenic activities [22]. They include the lignans, found in most plant foods but in the highest concentrations in flaxseed [23], which might contribute to the health effects of flaxseeds, and exhibit protective effects against hormone-related types of cancer like breast cancer [24]. Furthermore, they lower the risk of cardiovascular diseases [25] and obesity [26]. Many studies also have focused on the possible role of lignans from flaxseed that may prevent bone loss in postmenopausal women [27] or ovariectomized (OVX) model [28].

Traditional therapeutic agents for osteoporosis that stimulated bone formation may prevent further bone loss in established osteoporosis, but their costs are too high to benefit a large population in the developing countries. Consequently, there is increase request for developing "natural" products with less undesirable side effects that can reduce the need for drugs. This study was aimed to investigate the effect of avocado or flaxseed oils and their synergetic effect on bone heath and bone turnover in female osteoporotic rats.

MATERIALS AND METHODS

Plant Oils

Avocado and flaxseed oils were purchased from Agriculture Ministry, Cairo, Egypt.

Chemicals

Glucocorticoid (prednisolone) was purchased from Aventis Company, Cairo, Egypt. Calcium carbonate used as Ca supplement was purchased from El-Gomhoria Company, Cairo, Egypt.

Experimental Animals

Sixty female adult albino Wistar rats weighing 150±10g, supplied from the Breading Unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt) were used in this study. Rats were randomly housed individually in stainless steel cages with constant controlled environment; temperature 25^{o}C ± 5 ^{o}C, air humidity 55% ± 10% and 12/12 hrs light/dark were held. All rats were offered the balanced diet with drinking water *ad libitum* for a week for adaptation

Diet

The experimental diet used in the present study was the balanced diet prepared according to American Institute of Nutrition (AIN-93) [29] the oil content was modified according to the experiment.

Induction of Osteoporosis

Osteoporosis was induced in rats by daily oral dose of (6mg prednisolone/kg body weight) for 3 weeks [30].

Experimental Design

After acclimatization all rats were randomly divided into 2 main groups**:**

Group 1: 10 healthy rats fed on balanced diet and daily received 0.5ml distilled water orally

by stomach tube for the first three weeks (healthy control).

Group 2: 50 rats fed on balanced diet and after induction of osteoporosis, then rats were subdivided into 5 subgroups 10 rats for each.

10 rats fed on balanced diet (osteoporotic rats), 10 rats fed on balanced diet supplemented with Ca (7g/kg diet as calcium carbonate), 10 rats fed on balanced diet containing 4% Avocado oil and supplemented with Ca, 10 rats fed on balanced diet containing 4% flaxseed oil and supplemented with Ca and finally, 10 rats fed on balanced diet containing 4% of both oils and supplemented with Ca.

After 6 weeks of treatment all rats were sacrificed under ether anesthesia after 12 hrs fasting with water *ad libitum*. Blood samples were collected from hepatic portal vein for biochemical analysis. Femur bone was also separated and Kept at -8 ^{o}C. At the last day of experiment, 24 hrs urine samples were collected only for measuring Ca and P levels.

Biochemical Analysis

Serum Measurements

Serum samples were used for measurements of Ca and P levels by colorimetric method [31, 32]**.** Bone formation markers were also measured as osteocalcin (OC) level using (ELISA) kit [33] and alkaline phosphatase (ALP) activity by using colorimetric method [34] as well as, measuring bone resorption markers as parathyroid hormone (PTH) level using (ELISA) kit [35] and acid phosphatase (ACP) activity calorimetrically [36] Estrogenic effect of both oils was evaluated as E- estradiol level using ELISA kit [37].

Urine Measurements

At the end of experiment, 24 hrs urine samples were collected for measuring urine calcium and phosphorus levels calorimetrically as described in serum measurements [31, 32].

Bone Assessments

Right femur bones were cleaned, rinsed and washed by cold physiological saline solution then blotted on filter paper to remove water residue. The right femur scanned for determination of BMD and BMC using Dual-Energy X-ray Absorptiometry scanner (DEXA). Bone Ca and P levels were determined by digestion of bone tissue then measure element content by atomic emission spectrometric method by inductively coupled plasma mass mass (ICP.MS-MS).

Statistical Analysis

Data were statistically analyzed by Statistical Package for Social Science (SPSS) version 17.0. Values were presented as mean ± standard deviation (S.D.). Statistical differences between groups were performed using one-way Analysis of Variance (ANOVA), the mean difference was significant at the *($p < 0.05$)* level [38].

RESULTS AND DISCUSSION

Effect of feeding avocado or/and flaxseed oils on bone mineral density (BMD) and bone mineral content (BMC) in female osteoporotic rats

Dual-energy X-ray absorptiometry (DEXA) results presented in Table-1 showed that GC treatment for osteoporotic rats caused a significant reduction in BMD and BMC by 27.27% and 55.17% respectively compared to healthy rats. It was appeared that the administration of GC induced an increase in bone resorption, which results in a decrease in BMD, BMC and induction of osteoporosis.

Table-1: Effect of feeding avocado or/and flaxseed oils on bone mineral density (BMD) and bone mineral content (BMC) of osteoporotic female rats

Parameters **Groups**	**BMD** **(g/cm^2)**	**BMC** **(g)**
Healthy rats	**c**	**d**
Mean ±SD	0.132± 0.001	0.29± 0.02
Osteoporotic rats	**f**	**f**
Mean ±SD	0.096± 0.009	0.13± 0.02
% of change*	- 27.27	-55.17
Osteoporotic rats fed on Ca supplement	**e**	**e**
Mean ±SD	0.113± 0.006	0.21± 0.02
% of change*	- 14.39	-27.59
% of change**	17.71	61.64
Osteoporotic rats fed on Ca supplement and avocado oil	**d**	**c**
Mean ±SD	0.137± 0.004	0.33±0.03
% of change*	- 3.79	13.79
% of change**	32.29	153.85
Osteoporotic rats fed on Ca supplement and flaxseed oil	**b**	**b**
Mean ±SD	0.148± 0.001	0.50±0.03
% of change*	12.12	72.41

% of change**	54.17	284.62
Osteoporotic rats fed on Ca supplement and mix of oils	**a**	**a**
Mean ±SD	0.151± 0.006	0.56±0.01
% of change*	14.39	93.10
% of change**	57.29	330.77
LSD	0.001	0.01

- Values are represented for 10 rats in each group.
-There was no significant difference between means have the same letter in the same column *(p<0.05).*
* % of change from Healthy control, ** % of change from osteoporotic control

While, daily feeding osteoporotic rats Ca supplement with either avocado oil or/and flaxseed oil caused marked rise in the values of BMD and BMC compared to osteoporotic rats by {17.71%, 32.29%, 54.17% and 57.29 for BMD} and {61.64%, 153.85%, 284.62% and 330.77% for BMC} respectively *(P<0.05)*. It is clear that the treatment by Ca supplement with oils mix caused a highly significant increase in BMD and BMC which may decrease the risk of fractures than feeding single oil with Ca supplement or feeding Ca supplement alone, and this proved the effect of synergistic effect of oils on Ca availability in osteoporotic rats.

The result of the present study was in harmony with previous work revealed that GC therapy results in rapid loss of BMD, which is greatest in the first year of therapy and may be as high as 30% or more in the first 3–6 months depending on dose [39] also the study of Kozai *et al.,* who stated that predinsilone treatment (type of GC) significantly decreased the total BMC and BMD in the femoral metaphysis (-16% and -13%), respectively; (*P < 0.01*). The cortical BMC and bone area in the femoral diaphysis were also decreased significantly by prednisolone treatment (-9% and -8%, respectively; (P < 0.01). while, BMD in the femoral diaphysis was non-significantly decreased by (-1%) [40].

On the other hand, Watkins *et al.*, stated that higher femur BMD was observed with a low dietary ratio of n-6/n-3 poly unsaturated fatty acids (PUFAs). Whereas, growing female rats fed alfa lineolenic acid (ALA) rich flaxseed oil had higher whole-body BMC and BMD compared to animals fed corn oil [41]. In addition to Weiler *et al.***,** study who showed that growing female Sprague–Dawley rats fed oil rich in the long chain n-3 PUFAs, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaeonic acid (DHA, 22:6n-3), increased long bone BMD and BMC compared to rats fed corn oil low in n-3 PUFAs [42]**.** Also, it was confirmed that Long-chain polyunsaturated fatty acids (LCPUFA), especially the omega-3 fatty acids such as EPA and DHA, are beneficial for bone metabolism. The cellular mechanisms of action of the LCPUFAs are complex and involve modulation of fatty acid metabolites such as prostaglandins, cytokines, growth factors, and some other molecular signaling pathways. n-3 PUFAs can regulate bone metabolism by decreasing the release of prostaglandin E_2 (PGE_2) and, in the most important osteoclast differentiation factor RANKL [43]. Furthermore, n-3 PUFAs may modulate the number of proinflammatory cytokines, increasing production of insulin like growth factor-1(IGF1) and improving Ca accretion in bone [44]**.** That may explain the synergetic effect of both avocado and flaxseed oils that contain adequate amount of n-3 PUFAs.

Influence of feeding diet containing avocado or/and flaxseed oils on calcium (Ca) and phosphorus (P) levels in osteoporotic female rats

Induction of osteoporosis by prednisolone treatment showed statistically *(P<0.05)* significant reduction in serum Ca level (9.84±0.30 *vs* 10.48±0.82), and P level (4.25±0.18 *vs* 8.26±0.59) compared to healthy group. But after feeding osteoporotic rats Ca supplement only, there was an insignificant *(P>0.05)* increment in the Ca level by 4.17% (10.25±0.66) and significant *(P<0.05)* increment in P level by 73.41% (7.37±0.18) compared to osteoporotic control. While, feeding osteoporotic rats Ca supplement with either avocado or flaxseed oil or Ca supplement with both oils showed significant increment in serum Ca level (10.53±0.31), (10.56±0.30) and (10.55±0.34), respectively and also significant *(P<0.05)* increment in serum P level (7.28±0.52), (7.33±0.17) and (8.04±0.07) compared to osteoporotic group.

While comparing urine Ca and P levels of osteoporotic rats with normal control, it was clear that the Ca and P levels of osteoporotic group were significantly *(p<0.05)* increased (64.33±1.25 and 62.56±1.90) by 40.34% and 42.90% respectively. But when feeding osteoporotic rats Ca supplement alone, Ca supplement with either avocado oil or flaxseed oil, or Ca supplement with oils mix a marked decrease in Ca and P levels compared to osteoporotic control consuming balanced diet plus water only were recorded, the values were {50.29±1.10, 33.34±2.31, 35.86±0.38 *vs* 64.33±1.25} for Ca level, {50.01±1.07, 50.59±1.01, 42.00±0.24 *vs* 62.56±1.90} compared to osteoporotic control respectively *(p<0.05).*

With regard to results of bone Ca and P levels of osteoporotic rats which were {(10.34±0.40) and (8.79±0.11)}. It was observed that the treatment of osteoporotic rats by avocado or / and flaxseed oil led to a significant increment in Ca level that were (19.53±0.19), (19.71±1.36) and (19.90±0.50), by 88.88%, 90.62% and 92.46% respectively, These

increment were more significant than rats fed on Ca supplement only (17.13±0.80) by 65.67% compared to osteoporotic group *(P<0.05)*. On the other hand, P level was highly significantly increased in rats fed on Ca supplement with avocado oil, Ca supplement with flaxseed oil, Ca supplement with oils mix{12.08±0.59, 12.05±0.33, 12.16±0.07} by 37.43%, 37.09% and 38.34% respectively, whereas rats fed on Ca supplement only were increased by 32.88% (11.68±0.03) compared to osteoporotic rats *(P<0.05)*.

The results described in **(**Tables 2 and 3**)** illustrated that treatment by GC cause resorption of Ca and P from bone and increase their excretion in urine increasing the severity of osteoporosis that may lead to bone fractures. The systemic effects of GC on Ca metabolism were previously explained through inhibiting Ca resorption at the renal tubule and Ca absorption in the bowel through a vitamin D independent mechanism, decreasing transcellular active Ca transport and normal Ca uptake by brush-border membrane vesicles, and decrease synthesis of Ca-binding proteins as well that play an important role in bone mineralization [45]. Otherwise, the intestinal Ca absorption was impaired by GC therapy, it seems logical to increase intake Ca supplement or in the diet, suggesting that Ca supplement alone is not sufficient to prevent rapid bone loss in patients starting high-dose of GC [46]. The finding of this study revealed GC induced osteoporosis caused a significant reduction in serum Ca and P levels, and that was confirmed by the findings of other studies recorded that the levels of serum Ca and P were decreased in the osteoporotic rats [47, 48].

Table-2: Effect of feeding diet containing avocado or/and flaxseed oils on Calcium (Ca) level in serum, urine and bone of osteoporotic female rats

Parameters **Groups**	**Serum Calcium (mg/dl)**	**Urine Calcium (mg/dl)**	**Bone Calcium (g%)**
Healthy rats	**a**	**d**	**c**
Mean ±SD	10.48± 0.82	45.84±2.34	15.31± 0.49
Osteoporotic rats	**b**	**a**	**d**
Mean ±SD	9.84± 0.30	64.33± 1.25	10.34± 0.40
% of change*	- 6.11	40.34	- 32.46
Osteoporotic rats fed on Ca supplement	**a,b**	**b**	**b**
Mean ±SD	10.25± 0.66	52.62± 1.57	17.13± 0.80
% of change*	-2.19	14.80	11.88
% of change**	4.17	- 18.20	65.67
Osteoporotic rats fed on Ca supplement and avocado oil	a	**c**	**a**
Mean ±SD	10.53± 0.31	50.29± 1.10	19.53 ± 0.19
% of change*	0.48	9.71	27.56
% of change**	7.01	- 21.82	88.88
Osteoporotic rats fed on Ca supplement and flaxseed oil	a	**f**	**a**
Mean ±SD	10.56± 0.30	33.34± 2.31	19.71± 1.36
% of change*	0.76	-27.27	28.74
% of change**	7.32	-48.17	90.62
Osteoporotic rats fed on Ca supplement and mix of oils	**a**	**e**	**a**
Mean ±SD	10.55± 0.84	35.86± 0.38	19.90 ± 0.50
% of change*	- 5.25	- 21.77	29.98
% of change**	0.91	- 44.26	92.46
LSD	0.54	1.66	0.73

- Values are represented for 10 rats in each group.

-There was no significant difference between means have the same letter in the same column *(p<0.05)*

* % of change from Healthy Control, ** % of change from osteoporotic Control

Table-3: Effect of feeding diet containing avocado or/and flaxseed oils Phosphorous (P) in serum, urine and bone of osteoporotic female rats

Parameters Groups	Serum P (mg/dl)	Urine P (mg/dl)	Bone P (g%)
Healthy rats	**a**	**d**	**a,b**
Mean ±SD	8.26± 0.59	43.78± 0.35	11.88± 0.10
Osteoporotic rats	**c**	**a**	**d**
Mean ±SD	4.25± 0.18	62.56± 1.90	8.79± 0.11
% of change*	-48.55	42.90	- 26.01
Osteoporotic rats fed on Ca supplement	**b**	**b**	**b**
Mean ±SD	7.37± 0.18	59.26± 1.54	11.68± 0.03
% of change*	- 10.77	35.36	- 1.68
% of change**	73.41	- 5.27	32.88
Osteoporotic rats fed on Ca supplement and avocado oil	**b**	**c**	**a**
Mean ±SD	7.28± 0.52	50.01±1.07	12.08± 0.59
% of change*	- 11.86	14.23	1.68
% of change**	4.25	- 20.06	37.43
Osteoporotic rats fed on Ca supplement and flaxseed oil	**b**	**c**	**a**
Mean ±SD	7.33± 0.17	50.59± 1.01	12.05± 0.33
% of change*	- 11 26	15.56	1.43
% of change**	71.29	- 19.13	37.09
Osteoporotic rats fed on Ca supplement and mix of oils	**a**	**e**	**a**
Mean ±SD	8.04± 0.07	42.00± 0.24	12.16± 0.07
% of change*	- 2.66	- 4.07	2.36
% of change**	89.18	- 32.86	38.34
LSD	0.35	1.19	0.29

- Values are represented for 10 rats in each group.

-There was no significant difference between means have the same letter in the same column *(p<0.05)*

* % of change from Healthy Control, ** % of change from osteoporotic Control

Other study explained the role of avocado in ameliorate the osteoporosis as its high nutritive value with mineral content including abundant quantity of potassium,, P, Ca, Magnesium and others, and its high content of vitamins and antioxidant which have greater importance for overall bone health [49]. Moreover, flaxseed oil have high content of ALA that inhibit PGE_2 and also contain lignans that have estrogenic properties that decrease bone resorption and increase bone mineralization [50].

Although feeding avocado or flaxseed oils may have immense benefits ranging from its nutritional potentials which is reflected in its mineral composition to its ability to scavenging free radicals as its content of phenols and flavonoids. The results of the present work also clarified the role of synergistic effect of avocado and flaxseed oils as oils mix that has a double effect on Ca and P bioavailability than feeding one single oil only.

The result of the current study go hand in hand with the study that showed a significant decrease in Serum and bone Ca and P levels in the osteoporotic rats compared to the untreated controlled rats. While osteoporotic groups treated with Ca carbonate had significant increase in levels of Ca and P in both serum and bone, moreover, treatment of osteoporotic rats with Ca carbonate supplement with mixture of thyme and rosemary herbs rich in phenolic compound (as the oils have) caused the highest significant increase in both mineral levels in serum and bone. These changes started to ameliorate when the osteoporotic rats were treated with Ca carbonate or mixture of herbs, but the most amelioration was obtained when the rats received both Ca carbonate and mixture herbs [7].

Effect of feeding diet containing avocado or/and flaxseed oils on bone formation markers in osteoporotic female rats:

Results of Table-4 demonstrated the effect of feeding avocado and/or flaxseed oil on osteocalcin (OC) (ng/ml), alkaline phosphatase (ALP) (IU/L) levels as well as estradiol E_2 level (g/dl) of osteoporotic rats as indication of bone formation. The tabulated results demonstrated that there was a significant increment in OC levels of the osteoporotic rats by 6.53% compared to the healthy control group *(p<0.05)*. But after feeding osteoporotic groups avocado and / or flaxseed oils with Ca supplemented diet daily showed a significant decrement in OC level in osteoporotic rats. This decrement was 17.82± 0.40 for osteoporotic rats fed on avocado oil and was 17.69± 0.30 for osteoporotic rats fed on flaxseed oil. While in cases of osteoporotic rats fed on Ca supplement with oils mix, OC showed the highest significant decrement in their value, although, feeding Ca supplement only had less significant decrement in OC level (18.28±0.01) compared to osteoporotic control *(p<0.05)*.

Table-4: Effect of feeding diet containing avocado or/and flaxseed oils on osteocalcin (OC), alkaline phosphatase (ALP) and estradiol E_2 levels of osteoporotic female rats

Parameters Groups	Osteocalcin (ng/ml)	Alkaline phosphatase (IU/L)	Estradiol E_2 (g/dl)
Healthy rats	**c**	F	**a**
Mean ±SD	17.60±0.11	54.02± 3.14	107.54± 7.63
Osteoporotic rats	**a**	**A**	**e**
Mean ±SD	18.75± 0.21	87.77±1.18	48.81± 5.69
% of change*	6.53	62.48	- 54.61
Osteoporotic rats fed on Ca supplement	**b**	**B**	**d**
7667, m./Mean ±SD	18.28± 0.01	75.89±1.69	62.48± 3.25
% of change*	3.86	40.49	- 41.90
% of change**	- 2.51	- 13.54	28.01
Osteoporotic rats fed on Ca supplement and avocado oil	**c**	**C**	**c**
Mean ±SD	17.82± 0.40	71.27± 1.55	92.01± 2.21
% of change*	1.25	31.93	- 14.44
% of change**	- 4.96	- 18.80	88.51
Osteoporotic rats fed on Ca supplement and flaxseed oil	**c**	**D**	**b**
Mean ±SD	17.69±0.30	64.93± 4.30	101.71± 6.59
% of change*	-0.51	20.20	- 5.42
% of change**	- 4.21	- 26.02	108.38
Osteoporotic rats fed on Ca supplement and mix of oils	**d**	**E**	**d**
Mean ±SD	17.19± 0.33	57.46± 5.21	62.93± 4.98
% of change*	- 2.33	6.37	- 41.48
% of change**	- 8.32	-34.53	28.93
LSD	0.23	3.25	5.44

- Values are represented for 10 rats in each group.
-There was no significant difference between means have the same letter in the same column *(p<0.05)*
* % of change from Healthy Control, ** % of change from osteoporotic Control

When comparing ALP level of osteoporotic control with healthy control, it was clear that the ALP level of osteoporotic group increased significantly by 62.48% *(p<0.05)*. But when the osteoporotic rats feeding on avocado and/or flaxseed oils with Ca supplemented diet, there was a marked decrease in ALP level by 18.80%, 26.02% and 34.53% compared to osteoporotic control consuming balanced diet plus water only, the values were {71.27, 64.93, 57.46 *vs* 87.77} for osteoporotic groups fed on Ca supplement with avocado oil, Ca supplement with flaxseed oil and Ca supplement with oils mix compared to osteoporotic control respectively *(p<0.05).*

From the present results, it is also clear that estradiol E_2 in healthy group consuming balanced diet plus water only were (107.54±7.63). Osteoporosis induction by GC caused a statistically *(p< 0.05)* significant decrement in E_2 level which was (48.81±5.69) by 54.61%. Treatment of osteoporotic rats by avocado and/or flaxseed oil with Ca supplemented diet recorded a significant increase in their values when compared to osteoporotic control, the values were (92.01±2.21), (101.71±6.59) and (62.93±4.98), respectively. Whereas, feeding flaxseed oil for osteoporotic rats caused the highest increment of E_2 level compared to osteoporotic group.

It was cleared that feeding Ca supplement only has the least effect on all bone formation markers. While feeding either or both oils improve the efficiency of Ca supplement and increase Ca bioavailability by modulating the bone formation markers.

The obtained effect of prednisolone (GC) in the current study was in agreement with previous study revealed that low doses of GC (< 10 mg prednisolone equivalent per day) are, inducing osteoblasts differentiation by increased expression of mature bone markers, such as ALP and OC. While high doses or long-term GC therapy cause bone resorption and decrease BMD [51]. On the other hand, Kozai *et al*., found that the concentration of OC, a bone formation marker, was significantly decreased with prednisolone treatment compared to normal control [40]. GC inhibit expression of genes important for bone formation including those responsible for the production of collagen A1, transforming growth factor-β, fibronectin- and insulin-like growth factor-1 [52]**.**

Our finding agreed with Ribeiro *et al.*, that evaluated the bone parameters of rats treated with flaxseed diet. After 30 days of treatment, the female rats showed similar results to control group in OC level, BMD, BMC and bone area [53]. The mechanism of action of flaxseed on bone structure is complex and may related to several signaling pathways. ALA is related to the recruitment and maturation of pre-osteoblasts, thus promoting bone formation. As well as, ALA helps maintain OC level, secreted by the

osteoblast, and decrease RANK levels, a receptor found in osteoclast binding to RANKL. Therefore, ALA is associated with lower maturation, osteoclast lifespan and lower bone resorption [54]**.** That may also explain the effect of oils on OC level by its high content of ALA. The result also in good agreement with Oliveira *et al*.**,** [55] who found the administration of avocado/soybean unsaponifiables (a dose of 0.6 g per kg body weight on implant osseointegration in rat caused a significant increment in OC level compared to control healthy group.

With referred to the role of high content of phenols on avocado and flaxseed oils that significantly upregulated ALP gene expression and stimulated osteoblast differentiation, resulting in significantly increased bone mass [56]. Other previous work demonstrated that phenol compounds were able to enhance intestinal absorption of Ca, deposition of Ca ions in osteoblastic cells, and inhibition of osteoclast formation [57]**.** Moreover, Elkomy and Elsaid revealed the level of ALP had significant increment in osteoporotic group (65.00± 0.36) compared to the untreated controlled rats (47.66±0.44). While groups treated with Ca carbonate, herbs, Ca carbonate with herbs (rich in phenols and phytoestrogen), caused significant decrement in ALP level (55.38±0.55 52.60± 0.30 58.04±0.42) respectively. Also, OC level significantly increased in osteoporotic group (4.54±0.06) compared to healthy group (3.68±0.08), and treating by Ca carbonate and herbs have a positive effect [40]. It explained the more improvement in bone formation in rats fed on Ca supplement and single oil and the highest improvement with mix of oils by increasing Ca bioavailability.

Gong *et al*., showed that activation of GC receptor by dexamethasone induced the expression and activity of estrogen sulfotransferase, an enzyme important for the metabolic deactivation of estrogens, because sulfonated estrogens fail to activate the estrogen receptor. Treatment with GC lowered circulating estrogens, compromised uterine estrogen responses, and inhibited estrogen-dependent breast cancer growth [58]. Estrogen plays an important role in regulating bone metabolism and its deficiency was found to cause negative bone remodeling balance that augments bone loss and increases incidence of osteopenia. A number of mechanisms may contribute to this effect; however increased oxidative stress has a central role [59]. Furthermore, Phytosterols (oils rich with it), which have estrogen-like activity, enhance differentiation and proliferation of primary osteoblasts by increasing the mRNA expression of ALP and might modulate osteoclastogenesis via regulation of RANKL mRNA expression in bone cells [60].

Impact of feeding avocado and / or flaxseed oils on bone resorption in osteoporotic female rats:

Results of Table-5 clarified the effect of feeding avocado and / or flaxseed oil on acid phosphatase (ACP) (U/L) and parathyroid hormone (PTH) (pg/ml) as bone resorption markers in osteoporotic female rats.

Table-5: Effect of feeding diet containing avocado or/and flaxseed oils on acid phosphatase (ACP) and parathyroid hormone (PTH) of osteoporotic female rats

Parameters **Groups**	**ACP** **(U/L)**	**PTH** **(pg/ml)**
Healthy rats	**c**	**E**
Mean ±SD	30.03± 2.42	47.46± 1.21
Osteoporotic rats	**a**	**A**
Mean ±SD	93.12± 8.25	127.42± 5.98
% of change*	210.09	168.48
Osteoporotic rats fed on Ca supplement	**b**	**B**
Mean ±SD	38.32±4.07	60.94± 1.90
% of change*	27.61	28.40
% of change**	- 58.85	- 52.17
Osteoporotic rats fed on Ca supplement and avocado oil	**c**	**D**
Mean ±SD	29.49± 2.46	51.17± 1.45
% of change*	- 1.80	7.82
% of change**	- 68.33	- 59.84
Osteoporotic rats fed on Ca supplement and flaxseed oil	**c**	**D**
Mean ±SD	28.99± 3.95	52.33± 3.04
% of change*	- 3.46	10.26
% of change**	- 68. 87	- 58.93
Osteoporotic rats fed on Ca supplement and mix of oils	**d**	**C**
Mean ±SD	24.06± 0.65	56.35± 0.47
% of change*	- 19.88	18.73
% of change**	- 74.16	- 55.78
LSD	4.37	2.98

- Values are represented for 10 rats in each group.

-There was no significant difference between means have the same letter in the same column *(p<0.05)*

* % of change from Healthy control, ** % of change from osteoporotic control

Considering the results of ACP, the normal value was (30.03±2.42) in the healthy rats. Induction of osteoporosis by GC treatment caused significant increase in ACP (93.12±8.25) by 210.09%. However, feeding osteoporotic rats on diet containing Ca supplement with either avocado or flaxseed oil showed a statistically significant improvement in ACP activity with significant decrease in ACP (29.49±2.46 and 28.99±3.95) by 68.33% and 68.87%, respectively. While feeding both oils mix caused the highest effect on decreasing its value (24.06±0.65) by 74.16% which confirm the synergetic effect of avocado and flaxseed oils *($p<0.05$)*. With respect to the value of PTH activity of healthy and osteoporotic groups being (47.46±1.21) and (127.42±5.98). After feeding osteoporotic rats Ca supplement diet with either avocado or flaxseed oil or mix of both oils daily, there was a significant *($p<0.05$)* decrement in their values (51.17±1.45, 52.33±3.04 and 56.35±0.47) by 59.84%, 58.993% and 55.78, respectively compared to osteoporotic group. Osteoporotic rats fed on Ca supplement only had the least significant decrement of PTH by 52.17% compared to osteoporotic rats.

It was appeared from the current results that levels of serum ACP and PTH (bone resorption markers) in osteoporotic group were higher than those in treatment groups indicating the increase of bone turnover rate. Treatment with flaxseed and avocado oils decreased both of serum ACP and PTH levels, indicating a reduction in bone turnover.

Previous work showed spontaneous fluctuations in serum PTH levels in patients was found receiving therapy with GC (daily dosage 7.5mg of prednisone or dose equivalent of other corticosteroid) as compared with a control group. In the GC-treated group, the PTH tonic secretory rate was reduced 4.3±0.74 *vs* 8.8±1.4 pg/ml per min in controls. There was, however, an increase in the fractional pulsatile PTH secretion (42±8.2 *vs* 18.3±3.9 pg/ml per min) in GC-treated *vs* normal subjects. Mean overall PTH concentration, as well as mean integrated area, was similar among normal and GC-treated subjects [61]. Meanwhile, other study stated that osteoporosis caused elevation of PTH level [62]. Furthermore, Sobhani *et al.*, found that the administration of 0.2 mg/kg methylprednisolone acetate, 3 times/week for 4 weeks causes bone loss in rats. Bone resoeption marker ACP was significantly increased in osteoporotic rats compared to healthy rats (35.81*vs* 26.83) [63].

The study of Kim and Ilich concluded that for older adults (particularly postmenopausal women), supplementation with flaxseeds or flaxseed oil appears to have a marginal benefit to bone, possibly by inhibiting bone resorption [64]. Also, Elbostany *et al.*, found that levels of serum PTH significant deceased in treatment groups fed on basal diet supplemented with 10% flaxseed, 40% flaxseed bread, 0.1g lignin (5.92, 6.63 and 6.00) compared to control group (18.25) [23].

Moreover, Poulsen *et al.*, found that plasma concentration of intact PTH in the group treated by mix of omega-3 and omega-6 (present in high concentration in flaxseed and avocado oils) lower than osteoporotic rats. And so, increased dietary consumption of omega-3, and possibly some omega-6, long chain fatty acids may limit postmenopausal bone loss [65]. Previous work explained the effect of omega-3 Fatty acids in bone metabolism due tits anti-inflammatory effect that can lower the osteoclastic activity and reduce bone resorption [66].

Despite avocado oil is recently used as dietary supplement, it has grateful role as a natural alternative tool in medical treatment of secondary osteoporosis as shown by our results that revealed a nearly similar effect on flaxseed oil in treatment of osteoporosis, but the mix of oils had a more better effect on decreasing bone resorption and increasing bone formation which showed the role of synergistic effect on Ca bioavailability.

CONCLUSION

In conclusion, our study confirmed that combination of Ca supplement with either flaxseed oil or avocado oil can modulate the severity of secondary osteoporosis induced by glucocorticoid treatment. This improvement evident by significant elevation in bone formation biomarkers and significant reduction in bone resorption markers that increase both Ca and P bioavailability and caused significant increase in BMD and BMC. The improvement of osteoporosis reaches the highest level by using diet containing mixture of both oils with Ca supplement that revealed the synergetic effect of both flaxseed and avocado oils.

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