Role of Flaxseeds in Improving Bone Health of Female Rats with Osteoporosis by Glucocorticoids

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Abstract:

Glucocorticoids (GCs) have been widely available used to treat many of chronic diseases and the long-term use or high dose of glucocorticoids can be induced bone tissue damage leading to secondary osteoporosis. This study aimed to assess the effect of flaxseeds on bone biomarkers changes in osteoporotic rats. Forty adult albino female rats weighing (180±5g) were used in this study. Rats were divided into five equal groups, (8 rats each). The first group was negative control group which was fed standard diet. From 2 to 5 group were given prednisolone by oral gavage at dosage of 10 mg/kg body weight daily for 21 days. The second group was fed on standard diet as positive control group, the third, fourth and fifth groups were fed on standard diet containing 5, 7.5 and 10% of flaxseed powder for 8 weeks respectively. Supplementation osteoporotic rats with flaxseed led to improving serum calcium, ionized calcium, phosphorus, magnesium, alkaline phosphatase activity, bone-specific alkaline phosphatase activity, tartrate-resistant acid phosphatase activity, estrogen, calcitonin, osteocalcin, parathyroid hormone and lipid levels as compared to positive control. Moreover, femur weight, length, calcium, phosphorus, magnesium and BMD in osteoporotic rats supplemented with flaxseed were significantly increased as compared to osteoporotic rats. This study concluded that flaxseed is effective to improving bone health in osteoporotic rats.

Keywords: Bone mineral density, Osteocalcin, osteoporotic rats, calcitonin

Introduction:

Osteoporosis represents the main public health problem which affects millions of people around the world and its frequency increases by age **(Jalili *et al*., 2007)**. About 200 million persons worldwide are suffering from osteoporosis-related health complications, 33% of women and 20% of men over 50 years of age have at high risk of fractures due to osteoporosis **(Akarirmak, 2018)**. Osteoporosis could be defined as a metabolic bone disease or advanced systemic skeletal disease recognized by low bone mass and microarchitectural impairment of bone structure with an increase in bone fragility, reduced bone quality leading to a high risk of fracture **(Jeremiah *et al*., 2015).** Osteoporosis as a results from an imbalance of uncoupling of osteoblasts and osteoclasts with an increase in osteoclasts activity causing bone loss. The most common type of osteoporosis caused by drugs as Glucocorticoids -induced osteoporosis (GIOP) **(Maricic *et al*., 2018)**.

Glucocorticoids (GCs) have been widely available used to treat many of chronic diseases such as rheumatoid arthritis, gastrointestinal, pulmonary and autoimmune diseases **(Wang *et al*., 2020 and Seibel *et al*., 2013)** due to their potent anti-inflammatory and immunosuppressive activities **(Rizzoli and Biver, 2015)**. GCs are one of the most rife reason of secondary osteoporosis, because of the long-term use or big dose of glucocorticoids can induce bone tissue damage through decrease bone mineral density, increase bone resorption induce negative calcium balance and cause a high risk of fractures leading to GC-induced osteoporosis **(Wang *et al*., 2019)**. The primary reason for GC-induced osteoporosis attributed to the apoptosis of osteoblasts and osteocytes. Glucocorticoids damage osteoblast function and cause the apoptosis of both osteoblasts and osteocytes, resulted in an inhibition of bone formation **(Weinstein, 2011)**.

Diet plays a vital role in the skeletal growth and protection of bone health throughout life. Poor bone health will lead to an increased risk of osteoporotic fracture **(Ioannou and Boyko, 2013)**. Flaxseed (*Linum usitatissimum*) is a folk traditional food and medicament, recently it is used as functional food due to unique nutrient content. It is high in ω-3 fatty acid, α-linolenic acid, short chain PUFA, phytoestrogenic, lignans, proteins, soluble and insoluble fibers and antioxidants **(Marpalle *et al*., 2014 and Hussan *et al*., 2012)**. Flaxseed shows potential health benefits such as anti-diabetic, antioxidant, anti-inflammatory activity which reduce risk of cancer, cardiovascular, renal disorders **(Katare *et al*., 2012)**, osteoporosis **(Wahba and Al-Zahrany, 2013)** and menopausal symptoms **(Tzang *et al*., 2009)**. This is due to its high content of α -linolenic acid (ALA), dietary fibres and lignans **(Lowcock *et al*., 2013)**. Flaxseed is the richest sources of lignans (phytoestrogens) which plays an important role in the prevention of hormone-associated osteoporosis **(Singh *et al*. 2011)**. Also, flaxseed oil plays a vital role in the prevention of bone loss and regulation of bone metabolism leading to prevention of osteoporosis due to its high content of polyunsaturated fatty acids (PUFAs) **(Elbahnasawy *et al*., 2019 and El-Saeed *et al*., 2018)** where was found the omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are effective to increasing the bone formation and skeleton functions **(Iolascon *et al*., 2017)** by controlling of osteoblastogenesis and inhibition of bone resorption **(Kelly *et al*., 2013)**.

Due to the lack of data of effective treatments for secondary osteoporosis induced by the long-term of glucocorticoid thereby .Thus, the main objective of the present study was to evaluate the role of flaxseed in reducing development of bone loss and osteoporosis in GC-treated rats.

Materials and methods:

Materials

Flaxseed (*linum usitatissimum*) was procured from the Agriculture Research Center, Giza, Egypt. Glucocorticoid (prednisone acetate) was purchased from pharmacy Shibin El-Kom, Menoufia, Egypt. Kits were obtained from Alkan Medical Company, St. El Doky, Giza, Egypt. All other chemicals and reagents used in this experiment were purchased from El-Gomhoreya Company, Cairo, Egypt. Forty adult albino female rats, Sprague –Dawley strain weighing (180 ± 5 g) used in this study were purchased from Faculty of Medicine, Benha University, Egypt. The animals were acclimatized for 7 days in our animal house (Regd. No MUFHE / F /NFS / 5 / 22) before dietary manipulation. They were housed two per cage in an air- conditioned room (22± 2°C) with 12 h light/ dark cycle and had free access to standard pellet diet and water. All the procedures were performed in accordance with the Institutional Animal Ethics committee.

Methods

Preparation of Flaxseed flour

The seeds were cleaned manually to be them free of dust, carefully washed several times with distilled water, dried at room temperature for 3 days. The seeds were ground by (Moulinex miller, France) to be a fine powder then put in polyethylene bags and stored in a freezer until used.

Chemical analysis

Moisture, Protein, fat, fiber and ash contents were determined in flaxseed powder according to **AOAC (2012)**. The carbohydrate were calculated by difference. Omega-3 fatty acids was determined acorrding to the method of **Zahran and Tawfeuk (2019)**.

Experimental design

Rats were housed in environmentally controlled atmosphere and were fed standard diet for one week as an adaptation period according to AIN-93 guidelines (**Reeves *et al.*, 1993**) in animal laboratory in the Faculty of Home

Economics, Department of Nutrition and Food Science, Shibin El-kom, Menoufia, Egypt . After acclimatization period rats were divided into five equal groups, (8 rats each). The first group was negative control group which was fed standard diet. From 2 to 5 group were given prednisolone (Glucocorticoid) by oral gavage at dosage of 10 mg/kg body weight daily for 21 consecutive days according to **Kaczmarczyk-Sedlak *et al*. (2012)** to induce osteoporosis**.** The second group was fed on standard diet as positive control group, the third, fourth and fifth groups were fed on standard diet containing 5, 7.5 and 10% of flaxseed powder for 8 weeks, respectively. Moral guidelines for the care and treatment of animals were carefully followed in accordance with the rules of the Egyptian animal protection. At the end of experimental period, blood samples were centrifuged to obtain serum. Serum was kept frozen at −20°C for biochemical analysis. Left femur from each rat were removed and stored at – 4 Cº until estimation of bone mineral density (BMD). And after that the left femur from each rat were cleaned of soft tissue, weighted and used in determination of bone mineral contents.

Bone mineral density

Bone mineral density (BMD) of the left femur of each rat were measured by dual x-ray absorptiometry (DXA; model DCS-600A; Aloca, Tokyo, Japan).

Determination bone contents (calcium, phosurous and magnesium)

The left femurs and tibias were dried at 80°C for 18 hours to evalute bone weight, and then ashed at 600°C for 24 hours. Ashed samples were dissolved in 4 ml of 0.1 NHCl, and then diluted apppropriately with distilled water for atomization. Bone calcium, phosurous and magnesium were analyzed using Flame Atomic Absorption spectrophotometry (Model 5100 PC, Perkin-Elmer, Norwalk, CT) acorrding to the method described by **Fraser *el al.* (1986)**.

Biochemical assays

Serum calcium, ionized calcium, phosphorus and magnesium were estimated by colorimetric methods described by **Gindler and King (1972)**; **Boink *et al.* (1991); Maria *et al.* (1983) and Abdulsahib (2011),** respectively. Osteocalcin, estrogen, calcitonin, parathyroid hormone (PTH), bone alkaline phosphatase (BALP), alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities were determined in serum by using kits according to **Lee *et al.* (2003); Owens and Ashby (2002); Daumerie *et al.* (2013); Paik *et al*. (2010)**; **Rosalki *et al.* (1993); Varley *et al*.(1980) and Smith *et al.* (2005),** respectively. Serum triglyceride (TG), total cholesterol and high density lipoprotein (HDL-c) were measured according to the methods of **Fossati and Prencipe (1982); Allain *et al.* (1974), and Demacker *et al*. (1980),** respectively. Very low density lipoprotein cholesterol (VLDLc) and low density lipoprotein cholesterol (LDLc) were calculated according to the methods of **Lee and Nieman (1996)** as follows:

VLDLc = TG/5 and LDLc = Total cholesterol - (HDLc + VLDLc).

Statistical Analysis

Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program **(Artimage and Berry, 1987).**

Results and discussion:

Major chemical constituents of flaxseed were presented in Table (1). Data showed that carbohydrate (25.9%), fat (38.24%), protein (20.45%), Ca (240.75 mg/100g), P (629.67 mg/100g), Mg (420.13 mg/100g) and omega-3 fatty acids (202.4 mg/g) were high in flaxseed while, moisture (4.47%), ash (3.35%) and fiber (8.30%) were low. **Hamouda (2019)** indicated that the moisture, protein, fat, carbohydrates, ash and fiber of flaxseed were 6.50, 24.4, 35.12, 28.2, 2.63 and 3.15%

respectively. In comparison with **Bernacchia *et al*. (2014)** who showed that flaxseed contained Ca (236 mg/100g), P (622 mg/100g), Mg (431mg/100g). Also, **Elbahnasawy *et al.* (2019) who** found that the major dietary fatty acid found in flaxseed oil was omega-3PUFA at more than 40%.

Table (1): Major chemical constituents of flaxseed.

Chemical Constituents	Contents
Moisture (g/100 g)	4.47 ± 2.04
Carbohydrate (g/100 g)	25.19 ±1.8
Fat (g/100 g)	38.24 ± 1.98
Protein (g/100 g)	20..45 ± 0.92
Ash (g/100 g)	3.35 ± 1.05
Fiber (g/100 g)	8.30 ± 0.93
Ca (mg/100 g)	240.75 ± 2.5
P (mg/100 g)	629.67 ± 3.2
Mg (mg/100 g)	420.13 ± 2.7
Omega-3 fatty acids (mg/g)	202.41 ± 3.57

Each value in the table is the mean ±standard deviation

Table (2) shows serum levels of calcium (Ca), ionized calcium, phosphorus (P) and magnesium (Mg) of the control and osteoporotic rats. It was found that the osteoporotic rats had a significant decrease in the serum levels of Ca, ionized Ca, P and Mg as compared to the normal rats. This decrease in serum minerals levels due to glucocorticoids (GCs) which induce disturbance of Ca and P homeostasis through prevent absorption from intestinal tract, block renal reabsorption resulted in urinary excretion of Ca and P and may reduce intracellular Ca and P **(Suarez- Bregua *et al*., 2018 and Griel *et al*., 2007)**. These results matched with that of **Elbahnasawy *et al*. (2019)** and **Al-Bogami *et al*. (2016)** they found that osteoporotic rats had a significant reduction in serum levels of Ca and P, while the serum Mg levels in osteoporotic rats showed no significant changes when compared with the normal rats.

Supplementation osteoporotic rats diet with different concentration of flaxseed (5, 7.5 and 10%) significantly ($P \leq 0.05$) improved the serum levels of Ca, ionized Ca, P and Mg

as compared to 0% flaxseed. This improvement are mainly attributed to the high content of omega-3 fatty acids, alpha-linolenic acid (ALA), lignans, Ca, P and Mg in flaxseed. **Heaney *et al*. (2005)** found that omega-3 fatty acids increased calcium absorption in humans and kept Ca and P homeostasis, whereas omega-3 fatty acids improve osteoblast activity and inhibit osteoclast activity **(Watkin *et al*., 2003)**. Moreover, the highest improvement of Ca, ionized Ca, P and Mg were observed in osteoporotic rats feeding with 10% flaxseed by 49.26, 37.11, 25.42 and 73.39% respectively when compared with 0% flaxseed. As there were no significant change ($P > 0.05$) in the levels of Ca, ionized Ca, P and Mg among osteoporotic rats feeding 10% flaxseed and normal rats. These results coincided with the findings of **Boulbaroud *et al*. (2008)** they reported that consumption of whole flaxseed (5, 50 and 100%) for 3 months resulted in significant increase in urinary excretion of Ca and P thus showing an increased of osteoblastic activity and decline of osteoclastic activity. Treatment osteoporotic rats with flaxseed oil led to improvement in the levels of Ca, P and Mg **(Al-Bogami *et al*., 2016).** Flaxseed is a rich source of alpha-linolenic acid (ALA), which promote Ca absorption in the gut, enhancement mineral deposition in bones **(Pessanha *et al*., 2016 and Florencio-Silva *et al*., 2015)**.

Table (2): Serum levels of Ca, iomized Ca, P and Mg of the control and osteoporotic rats.

Groups / Variables	Normal rats	Osteoporotic rats			
		0%flaxseed	5%flaxseed	7.5%flaxseed	10%flaxseed
Ca (mg/dl)	10.57 ± 0.57^{a}	6.76 ± 0.18^{d}	7.79 ± 0.21^{c}	9.06 ± 0.17^{b}	10.09 ± 0.28^{a}
Ionized Ca (mmol/L)	1.38 ± 1.24^{a}	0.97 ± 0.04^{d}	1.11 ± 0.03^{c}	1.24 ± 0.05^{b}	1.33 ± 0.03^{ab}
P (mg/dl)	8.54 ± 0.43^{a}	6.57 ± 0.14^{d}	7.18 ± 0.18^{c}	8.1 ± 0.09^{b}	8.24 ± 0.07^{al}
Mg (mg/dl)	2.21 ± 0.12^{a}	1.24 ± 0.06^{d}	1.49 ± 0.1^{c}	1.97 ± 0.11^{b}	2.15 ± 0.05^{a}

Values are expressed as means ± SD; Means in the same raw with different letter are significantly different ($P \leq 0.05$).

Serum levels of bone turnover parameters in the control and osteoporotic rats were presented in Table (3). The normal bone turnover based on the balance among osteoblastic bone forming and osteoclastic bone resorption **(Hadjidakis and Androulakis, 2006)**. Data in Table (3) illustrated the levels of alkaline phosphatase (ALP) and bone specific alkaline phosphatase (BALP) in osteoporotic rats were significantly lower ($P \leq 0.05$) than that of normal rats while, tartrate-resistant acid phosphatase (TRAP) had an opposite trend. These results reflect excess of bone resorption due to the activity of osteoclasts and increase its number. Glucocorticoids (GCs) therapy can breed an imbalance between bone formation and bone resorption leading to bone loss **(Weinstein, 2011)** whereas GCs can cause activation of apoptosis in osteoblastic bone formation and elevation in osteoclasts activity **(Chang *et al*., 2009)**. The obtained results had the same trend of **Wang *et al*. (2020)** they reported that glucocorticoid-induced osteoporosis rats had a significant decrease in serum ALP activity and a significant increase in the levels of TRAP. Also, serum ALP as a marker of bone formation and BALP as a biomarker of osteoblast differential and bone metabolism **(Peng *et al*., 2010 and Hatakeyama *et al*., 2019)** were significantly reduced in osteoporotic rats while TRAP had an opposite trend compared to normal control **(Eskandarynasab *et al*., 2020 and Xia *et al*., 2019)**.

Feeding flaxseed (5, 7.5 and 10%) to osteoporotic rats resulted in elevation ($P \leq 0.05$) in the activity of ALP and BALP accompanied by reduction ($P \leq 0.05$) in TRAP activity as compared with 0% flaxseed. This effect may be due to the activity of flaxseed in decline the osteoclast number and an increase the osteoblast count, whereas flaxseed is a very rich dietary source of phytoestrogen lignans, omega-3 fatty acids and alpha-linolenic acid **(Koetz *et al*., 2012; Ivanova *et al*., 2011 and Singh *et al*., 2011)** Also, It was observed that feeding osteoporotic rats on diet supplemented with 10% of flaxseed was more effective ($P \leq 0.05$) in increasing ALP and

BALP activities than those supplemented with 5 and 7.5% of flaxseed. This may be due to high content of lignans, omega-3 fatty acid and α-linolenic acid in flaxseed (10%) than 5 and 7.5% of flaxseed. These results matched with that of **AL-Bogami *et al.* (2016)** they found that administration osteoporotic rats to flaxseed oil led to elevate ALP activity. The omega-3 fatty acids elevate the activity of alkaline phosphatase (ALP) and bone formation markers **(Watkins *et al.*, 2001)**, where it was found that omega-3 PUFAs might efficiently and effectively increase skeleton functions and the bone formation rate **(Iolascon *et al.*, 2017)** through inhibit the action of osteoclasts and improve the activity of osteoblasts **(Elbahnasawy *et al.*, 2019)**. Tartrate-resistant acid phosphate (TRAP) was reduced ($p \leq 0.05$) by 24.59, 41.2 and 68.66% for osteoporotic rats feeding with 5, 7.5 and 10% of flaxseed, respectively. These results confirmed the results of **Boulbaroud *et al.* (2008)** they found that eating high diet in PUFA for 4 weeks decreased TRAP activity and return its near to normal levels in rats. Omega-3 fatty acids influence bone healthy by improving calcium balance and bone turnover through its effect on osteoclastic and osteoblastic differentiation and activity reported by **Ikpeama *et al.* (2014)** and **Lau *et al.* (2013)**. Moreover, Alpha linolenic acid (ALA) has a vital role in osteoblastogenesis and prevention of bone resorption **(Kelly *et al.*, 2013)** as well as **AbdelKarem *et al.* (2011)** showed that Lignans has an anabolic effect on bone metabolism and inhibited bone loss.

Table (3): Serum levels of bone turnover parameters in the control and osteoporotic rats.

Groups / Variables	Normal rats	Osteoporotic rats			
		0% flaxseed	5% flaxseed	7.5% flaxseed	10% flaxseed
Alp (u/L)	$127.33 \pm 4.^{a}$	77.16 ± 1.83^{d}	81.75 ± 3.31^{d}	98.66 ± 2.23^{c}	117.3 ± 4.1^{b}
BAlP (u/L)	421 ± 4.69^{a}	271.38 ± 3.8^{e}	314.01 ± 3.8^{d}	375.03 ± 4.0^{c}	410.37 ± 3^{b}
TRAP (mg/ml)	1.85 ± 0.17^{e}	8.01 ± 0.58^{a}	6.04 ± 0.24^{b}	4.71 ± 0.13^{c}	2.51 ± 0.18^{d}

Values are expressed as means ± SD; Means in the same raw with different letter are significantly different (P ≤0.05. AlP: alkaline phosphatase; BAlP: bone specific alkaline phosphatase; TRAP: tartrate-resistant acid phosphatase..

Data presented in Table (4) illustrated that serum levels of estrogen, calcitonin, osteocalcin and parathyroid (PTH) hormones in the control and osteoporotic rats. The level of estrogen, calcitonin and osteocalcin were the lowest (P≤ 0.05) in osteoporotic rats as compared to normal rats while PTH had an opposite trend. These results are similar to those reported by **Eskandarynasab *et al*. (2020)** they found that a significant reduction in osteocalcin level in osteoporotic rats. **Elbahnasawy *et al*. (2019)** observed that the prednisolone group (osteoporotic rats) had the highest (P<=0.05) PTH level in comparison to the normal rats. As injection with Gcs led to a very highly significant elevation in PHT level reported by **Al-Bogami *et al*. (2016)**. It has been observed that high levels of PHT lead to increasing bone resorption rate through transferring bone calcium into the blood to return the normal plasma calcium levels **(Elbahnasawy *et al*., 2019),** this is attribute to PHT hormone has an important role in regulating blood calcium and phosphor by improving intestinal calcium absorption and inhibition re-absorption of phosphor by proximal convoluted tubule as well as play a vital role in proliferation of osteoclasts and osteoblasts **(Chapurlat and Confavreux, 2016)**. Also, deficiency estrogen level in females raised the action PTH on bones resulting in lower BMD and bone resorption **(Krivosikov *et al*., 2010).** Decreased the level of calcitonin may be due to the calcium level is the principal stimulus for its secretion by C-cells, when blood calcium is reduced resulting in secretion deficiency of calcitonin **(Zaidi *et al*., 2002).**

As described in Table (4) no significant (P>0.05) differences were observed in the levels of estrogen and calcitonin between osteoporotic rats supplemented with 0% flaxseed and those supplemented with 5% flaxseed. The levels

of estrogen and calcitonin were significantly (P≤0.05) increased in osteoporotic rats supplemented with 7.5 and 10% flaxseed by 36.53, 58.19, 18.04 and 29.32%, respectively as compared to rats supplemented with 0% flaxseed. Supplementation osteoporotic rats with 10% flaxseed was more effective (P≤0.05) in increasing the level of estrogen and calcitonin than those supplemented with 7.5% flaxseed. Phytoestrogen have positively influence on bone health problems, where it has various estrogenic and antiestrogenic effects **(Anderson *et al*.,2013 and Usui, 2006)**. Phytoestrogens protect from bone loss through it looks to direct increase estrogen levels **(Rice and Whitehead, 2008)** and this is due to their structural similarity to estrogens which may help to exert estrogen like- activties **(Beral *et al*., 2002).** Estrogens are main promotes of bone formation, when the level of estrogen reduced in the body, lignans may work like weak estrogen these reported by **Chiang and Pan (2013)** and **Holick (2004)**. Also, **Word *et al*. (2001)** showed that lignans has potentially direct effects on bone tissue through interaction with the oestrogen receptor-B.

On the other hand, osteocalcin levels in osteoporotic rats supplemented with flaxseed was significantly (P≤0.05) elevated by increasing the level of flaxseed while, PTH had an opposite trend. The highest elevation of osteocalcin and the highest reduction in PTH were found in osteoporotic rats supplement with 10% flaxseed by 155.04 and 66.05% as compared to 0% flaxseed. This findings were similar to the previously reported by **Al-Bogami *et al.* (2016)** who concluded that feeding rats injected with Gcs to flaxseed oil resulted in decline in the level of PTH, as **Costa *et al*. (2016)** who found that flaxseed flour diet elevated serum osteocalcin. Moreover, it was noticed that no change (P>0.05) in the levels of osteoralcin and PHT among osteoporotic rats supplemented with 10% flaxseed and normal rats. **Lukas *et al*. (2011)** showed that feeding rats flaxseed oil, rich in ALA increased serum osteocalcin level which leads to stimulation bone

formation, where it was found that ALA maintains bone mass by raising Key transcription factors as osteocalcin, which improve preosteoblasts differentiation and bone formation **(Longo and Ward, 2016)**.

Table (4): Serum levels of estrogen, calcitonin, osteocalcim and Parathyroid hormones in the control and osteoporotic rats.

Groups / Variables	Normal rats	Osteoporotic rats			
		0%flaxseed	5%flaxseed	7.5% flaxseed	10% flaxseed
Estrogen (mg/ml)	67.36±1.8^{a}	39.75±3.21^{d}	42.83±3.47^{d}	54.27±1.98^{c}	62.88±1.4^{b}
Calcitonin (pg/ml)	45.69±3.3^{a}	31.04±2.13^{d}	33.68±1.56^{cd}	36.64±2.3b^{c}	40.14±2.8^{b}
Osteocalcin (pg/ml)	16.26±1.2^{a}	5.85±1.08^{d}	9.22+0.97^{c}	12.71±1.4^{b}	14.92±0.8^{a}
PTH (pg/ml)	303.13±6.25^{d}	932.75±10.72^{a}	667.25±12.7^{b}	489.5±12.15^{c}	316.63±9.88 ^{d}

Values are expressed as means ± SD; Means in the same raw with different letter are significantly different (P ≤0.05). PTH: Parathyroid hormones.

Results in Table (5) represent the serum levels of lipid profile in the control and osteoporotic rats, It was clear that osteoporotic rats presented an increase (P≤0.05) of cholesterol, triglycride, LDL.c and VLDL.c levels as compared to normal rats whereas, HDL.c had an opposite trend. This results showed lipid metabolic disorders with hypercholesterolemia and hyperlipidemia as a clear risk for osteoporosis in rats. The obtained results were in agreement with the findings of **Lee *et al*. (2020)** who revealed that the levels of total cholesterol and triglyceride raised in rats injected with dexamethasone indicating the, negative effect of glucocorticoid. Also, **Quinkler *et al*. (2017)** who showed that prednisolone treatment (3.6 mg/day) resulted in elevation in the levels of total cholesterol and LDL.c. Glucocorticoid increase total cholesterol and LDL.c as well as reduce TC/HDL.c ratios in human reported by **Choi and Seeger (2005)**.

A signification decrease in the level of the total cholesterol, TG, LDL.c and VLDL.c and an increase in the level of HDL.c were observed in osteoporotic rats received 5, 7.5 and 10% of flaxseed relative to osteoporotic rats received

0% flaxseed (positive rats). This hypolipidemic effect of flaxseed could be due to its high content of alpha linolenic acid, lignans, phytoestrogen, phenolic compound and soluble fiber **(Bloedon and Szapary, 2004** and **Kroliczewska *et al*., 2018)**. The obtained results agreed with that of **Hadi *et al*. (2020)** who found that flaxseed and its products improved liquid profile in human by reducing circulating total cholesterol, TG and LDl.c. Furthermore, the highest reduction in the levels of cholesterol, TG, LDL.c and VLDl.c were found in osteoporotic rats receiving 10% of flaxseed compared to other concentrations of flaxseed. However, there were no significant change ($p>0.05$) in the level of HDL.c among osteoporotic rats received 10% of flaxseed and normal rat. These results confirmed the findings of **Saxena and Katare (2014)** who noted that flaxseed intake resulted in a highly decrease in total cholesterol, triglycerides, LDL.c and VLDL.c levels. Also, **Cardozo *et al*. (2010)** showed that supplementation rats diet with 25% of flaxseed caused reduction in the level of triglyceride from 79.86 to 54.25 mg/dl and total cholesterol from 63.43 to 45.71 mg/dl without any change in HDL.c level.

Table (5): Serum levels of lipid profile in the control and osteoporotic rats.

Groups / Variables	Normal rats	Osteoporotic rats			
		0% flaxseed	5% flaxseed	7.5% flaxsed	10% flaxsed
Cholesterol (mg/dl)	81.61±2.41d	141.62±3.94a	129.52±1.74b	104.09±6.41c	87.11±2.54d
TG (mg/dl)	69.45±2.86e	111.003±1.44a	98.39±4.21b	83.21±2.73c	74.18±1.69d
HDl.c (mg/dl)	54.44±1.42a	39.02±2.16d	41.78±1.86c	46.54±0.62b	52.27±0.99a
LDl.c (mg/dl)	13.28±2.0e	80.4±5.73a	68.07±2.65b	40.92+6.35c	20.006+1.72d
VLDl.c (mg/dl)	13.89±0.57e	22.2±0.29a	19.68±0.84b	16.64+0.55c	14.84±0.34d

Values are expressed as means ± SD; Means in the same raw with different letter are significantly different ($P \leq 0.05$).

Femur bone marker levels in the control and osteoporotic rats is recorded in Table (6). The lowest values of weight, length, calcium, phosphorus, magnesium and bone mineral density (BMD) of the left Femur were observed in osteoporotic

rats (0% flaxseed) compared to normal rats. This decrease could be due to the GSc reduce the rate of calcification and osteogenesis by suppression of PTH **(Greer *et al*., 2008)**. Our findings were similar to the previously reported by **Elbahnasawy *et al*. (2019)** they found that administration of prednisolone (10 mg/kg/day) produced depletion in femur mass and bone mineral density (BMD) in rats. **Farlay *et al.* (2019)** showed that estrogen deficiency is associated with an unbalanced among formation and resorption in favour of bone resorption leading to bone loss. Also, Al-**Bogami *et al*. (2016)** showed that rats injected with GCs caused a very highly reduction in the level of Ca with accompanied highly decline in p level in the left femur of osteoporotic rats while there is no significant change in the level of Mg as compared to negative control rats.

On the other hand, it was mentioned that the values of femur weight, length, Ca, P, Mg and BMD of osteoporotic rats supplemented with flaxseed were significantly ($P<=0.05$) increased by increasing the level of flaxseed. **Al Bogami *et al*. (2016)** reported that treatment osteoporotic rats with flaxseed oil resulted in deposition Ca, P and Mg in bone and this is due to flaxseed lignans and omega 3 fatty acid increase the rate of calcification and correction Ca and P level. Phytoestrogen can inhibit osteoporosis by suppressing the rate of bone resorption and improvement the bone formation rate **(Chiang and Pan, 2013)**. Omega-3 PUFA and alpha linolenic acid promote bone formation via inhibit lipid peroxidation that prevent osteoblastogenesis these reported by **Lukas *et al.* (2011)**. Supplementation osteoporotic rats diets with 7.5 and 10% of flaxseed did not significantly differ ($P>0.05$) in their effect on femur weight and length. However osteoporotic rats supplemented with 10% of flaxseed was more effective ($p\leq0.05$) in increasing Ca, P, Mg and BMD levels in femur than those supplemented with 5 and 7.5% of flaxseed. Also, there were no significant ($p>0.05$) change in femur weight, length, Mg and BMD among osteoporotic rats supplemented

with 10% of flaxseed and normal rats. **Elbahnasawy *et al.* (2019)** indicated that feeding rats flaxseed oil can play a role in the regulation of bone metabolism and prevention of bone loss. The omega-3 PUFAs might effectively increase skeleton functions and the bone formation rate by enhancing osteoblast activity and inhibit osteoclast activity **(Iolasscon *et al.*, 2017 and Watikins *et al.*, 2003)**. Moreover, **Ward *et al.* (2001)** found that exposure to purified lignan at the level present in 100g flaxseed/kg diet resulted in increased bone strength.

Table (6): Femur biochemical marker levels in the control and osteoporotic rats.

Groups / Variables	Normal Rats	Osteoporotic rats			
		0% flaxseed	5% flaxseed	7.5% flaxseed	10% flaxseed
Weight (g)	0.74±0.18a	0.45±0.13c	0.51±0.08bc	0.68±0.09ab	0.75±0.04a
Length (g)	3.94±0.21a	3.66±0.04b	3.78±0.02ab	3.86±0.03a	3.92±0.09a
Ca (mg/dl)	12.88±0.47a	6.90±0.26e	7.89±0.12d	8.86±0.42c	10.14±0.18b
P (mg/dl)	12.12±0.35a	7.84±1.75d	8.29±0.26d	9.77±0.27c	10.82±0.39 b
Mg (mg/dl)	2.52±0.13a	1.28±0.06d	1.58±0.09c	1.97±0.03b	2.40±0.04 a
BMD (g/cm2)	0.149±0.00a	0.072±0.006d	0.098±0.004c	0.132±0.005b	0.147±0.002a

Values are expressed as means ± SD; Means in the same raw with different letter are significantly different ($P \leq 0.05$).BMD: bone mineral density.

Conclusion:

Flaxseed supplementation for eight weeks showed potentially beneficial effects on bone metabolism and serum lipids in osteoporotic rats. So, data recommend that addition of flaxseed to daily diet during prolonged glucocorticoids therapy for its potential effect to reduce the risks of osteoporosis.

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دور بذور الكتان في تحسين صحة عظام إناث الفئران المصابة بهشاشة العظام بواسطة الجلوكورتيكويد

نجلاء على الشيخ
كلية الاقتصاد المنزلي- قسم التغذية وعلوم الأطعمة- جامعة المنوفية-شبين الكوم- مصر

الملخص العربي:

تستخدم الجلوكوكورتيكويدات (GCs) على نطاق واسع لعلاج العديد من الأمراض المزمنة ويمكن أن يؤدي الاستخدام طويل الأمد أو الجرعات العالية من الجلوكوكورتيكويد إلى تلف أنسجة العظام مما يؤدي إلى الإصابة بهشاشة العظام الثانوية. هدفت هذه الدراسة إلى تقييم تأثير بذور الكتان على تغيرات المؤشرات الحيوية للعظام في الفئران المصابة بهشاشة العظام.. تم اجراء الدراسة علي اربعون من اناث الفئران البيضاء البالغة وزنها (١٨٠±٥جرام) . قسمت الفئران إلى خمس مجموعات متساوية (٨ فئران لكل مجموعة). المجموعة الاولي مجموعة ضابطة سالبة تغذت علي الوجبة القياسية، أعطيت المجموعات من ٢ إلى ٥ بريدنيزولون جرعة يومية بالفم مقدارها ١٠ مجم / كجم من وزن الجسم لمدة ٢١ يوماً. غذيت المجموعة الثانية على الوجبة القياسية كمجموعة ضابطة موجبة ، أما المجموعة الثالثة والرابعة والخامسة فقد غذيت على وجبة قياسية تحتوي على ٥ و ٧.٥ و ١٠٪ من مسحوق بذور الكتان لمدة ٨ أسابيع على التوالي. أدى التدعيم بمسحوق بذور الكتان إلى تحسين الكالسيوم ،الكالسيوم المتأين ،الفوسفور ، الماغنسيوم ، نشاط انزيم الفوسفاتيز القاعدي ، نشاط انزيم الفوسفاتيز القاعدي الخاص بالعظم، ونشاط إنزيم الفوسفاتاز الحمضي المقاوم للطرطرات ، الإستروجين ، الكالسيتونين ، الأوستوكالسين ، هرمون الغدة الجار درقية ،ومستويات الدهون بالسيرم مقارنة بالمجموعة الضابطة الموجبة. علاوة على ذلك ،زاد وزن عظم الفخذ ، الطول ، الكالسيوم ، الفوسفور ، المغنيسيوم و كثافة المعادن بالعظام في الفئران التي تعاني من هشاشة العظام والمدعمة ببذور الكتان مقارنة بالفئران المصابة بهشاشة العظام.،وقد خلصت هذه الدراسة إلى أن بذور الكتان فعالة في تحسين صحة العظام في الفئران المصابة بهشاشة العظام.

الكلمات المفتاحية: كثافة المعادن بالعظام ، الأوستوكالسين ، الفئران المصابة بهشاشة العظام، كالسيتونين.