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The Potential Effect of Flax Seeds Against Osteoporosis in Experimental Female Rats

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Abstract: The main target of this study was to investigate the possible protective effect of flaxseed as phytoestrogens source on glucocorticoid-induced Osteoporosis in female rats. The alleviating roles of this plant on some other side effects of glucocorticoid therapy were also studied. Thirty female albino rats (Sprague Dawley strain) weighing 150 ± 10 g were used and randomly divided into five equal groups. The first group was fed on basal diet as a negative control, while other groups were fed on basal diets containing 100 mg of prednisone as a glucocorticoid/ kg diet (GCD) to induce bone loss. One of them, the positive control group was kept feeding on GCD alone, while others were fed on GCD containing 30, 50 and 70 g of flaxseed powder/kg diet. The experiment was lasted for 8 weeks. Feed intake, body weight gain and feed efficiency ratio were finally calculated. Bone mineral concentration (BMC), bone mineral density (BMD) as well as the concentrations of calcium and phosphorus in both serum and left femur bones was determined. Besides, the activities of serum transaminases, as the most specific markers of liver injury, were also determined. Moreover, the right femur bones were histopathologically examined. Results indicated that feeding GCD resulted in a significant decline in BMC, BMD and the concentrations of calcium and phosphorus in both sera and left femur bones which was supported by histopathological findings. Feed intake, body weight gains and hence feed efficiency ratio were also significantly reduced. Conversely, the activities of serum transaminases were significantly increased revealing a marked liver injury. Supplementation of GCD with the studied plant alleviated the marked lesion noticed in bone tissue and increased its mineralization and density. Feed intake and liver functions were improved in flaxseed –fed groups. Finally, dietary supplementation with flaxseed powder is recommended to prevent glucocorticoid-induced Osteoporosis and some other side effects. The supplementation was more potent in elevating bone mineral density.

Key words: Osteoporosis · Bone Mineral Density · Flaxseed · Glucocorticoid · Bone Histopathology · Phytoestrogens

INTRODUCTION

Glucocorticoids are commonly used in the cure of patients with long-lasting noninfectious inflammatory diseases, particularly asthma, chronic lung illness, rheumatoid arthritis, further connective tissue diseases and inflammatory intestine disease and in organ transplantation. Although, quick are several, bone loss in vertebral fractures is the most incapacitating impact [1].

Glucocorticoid-induced osteoporosis (GIOP) is the most widespread cause of secondary osteoporosis, the most frequent cause of osteoporosis by 50years of age and the most common iatrogenic reason. Previous and present exposure to glucocorticoids (GCs) raises the hazard of fracture and bone loss. Bone frailness in GIOP is described as quick bone loss resulting in the introduction of GCs, with an inconsistency between bone mineral density (BMD) and the risk of a break [2]. Van Staa *et al*. [3] reported that; most studies investigating the effect of the GCs on skeletal tissue contain patients of both genders and are harmonious in appearing that all patients, lad and old, male and female, are liable to the impacts of GCs on bone structure.

Osteoporosis, a skeletal disease distinguished by low bone mass density and microarchitectural damage of bone tissue, is a large-scale public health problem presently

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affecting over than 75 million people worldwide [4]. Eighty percent of persons who be ill with osteoporosis are women [5]. Females are more exposed to osteoporosis than males because of reduction in estrogen through menopause which results in a decline in bone-structure and augments in bone-resorption action. Estrogen is capable of quell production of proinflammatory cytokines like Inter lukin1 (IL-1), Inter lukin6 (IL-6), Inter lukin7 (IL-7) and Tumor necrosis factor Alfa (TNF-α). So these cytokines are high in postmenopausal females [6].

In the USA, the Food and Drug Administration has permitted many drugs for use in the protection and therapy of osteoporosis. Nevertheless, all of the currently obtainable agents have serious side effects that control their efficiency and emphasize the crucial need for new remedy options. One promising method is the development of substitutional (Non-pharmaceutical) methods to maintain bone, accompanied by the prevention and therapy of osteoporosis [7]. Accordingly, vegetal dietary supplements are ever more popular for females' health, especially for older women. The particular botanicals women take change as a purpose of age, certain estrogenic botanicals have also shown preventative impacts against osteoporosis [8].

Phytoestrogens are a fluctuated gathering of plant-inferred aggravates that basically or practically mirror mammalian estrogens and show potential advantages for human wellbeing [9].

As of now, four distinct groups of phenolic mixes delivered by plants are considered phytoestrogens: the isoflavonoids, stilbenes, lignans and coumestans. Various classes of phytoestrogens and assorted mixes inside each class influence the estrogen-interceded reaction in various ways [10].

For a considerable length of time, flax seeds (Linum usitatissimum) were suggested in the human eating routine, on account of their high substance of parts helpful for human wellbeing. Other than polyunsaturated unsaturated fats, they contain generally high amounts of secoisolariciresinol diglucoside (SDG), phenolic acids and flavonoids. Flax oil contains high amounts of the fundamental polyunsaturated unsaturated fats alpha-linolenic corrosive (ALA) and linoleic corrosive (LA). It has been generally demonstrated that an abnormal state of ALA in the eating regimen can diminish the danger of malignancy and cardiovascular sicknesses and breaking point the creation of arachidonic acids and other expert incendiary eicosanoids. The linseed unsaturated fats have been accounted for to influence cytokine gene expression. For instance, calves enhanced with flax oil (yet not angle oil) would, in general, demonstrate a lessening in the declaration of IL-4and IL-8 qualities [11]. Flaxseed is viewed as one of the most extravagant wellsprings of lignans phytoestrogens [12].

Several studies have developed techniques to identify and quantify phytoestrogens in flaxseed however restricted clinical investigations have been directed on the phytoestrogens in flax and their job in human wellbeing. From the past information our hypothesis is phytoestrogens prevent glucocorticoid-induced osteoporosis and some other side effects. Thus, in this study, we are trying to assess the job of flaxseeds as source of phytoestrogens in preventing glucocorticoid-induced osteoporosis and improving serum calcium, phosphorus and other related parameters.

MATERIALS AND METHODS

Materials: Thirty normal non-pregnant female albino rats of *Sprague dawley* strain (8 weeks old) weighing 150 ± 10 g were obtained from the laboratory animal colony Ministry of Health and Population, Helwan, Egypt.

Flaxseeds were obtained from the Agriculture Seeds, Herbs and Medicinal Plants Company, Cairo, Egypt.

Prednisone acetate, casein and cellulose powder were obtained from El-Sharqiya Co., while vitamin and salt mixture was from Adwiya Co., Kafr El-Zayat, Egypt.

Corn oil and corn starch were purchased from local market, Tanta City, Gharbia, Governorate, Egypt.

Experimental Design: Female albino rats *Sprague dawley* Strain (30 rats) weighing (150 ± 10 g.) were housed in well-aerated cages under a hygienic condition and fed on the basal diet for one week for adaptation *ad libitum* in the animal house of Faculty of Home Economics, Al-Azhar University.

Diets were presented to rats in special non – scattering feeding cups to avoid loss of food and contamination. Also, tape water was provided *ad libitum* and checked daily.

The basal diet was composed of 14 % casein (>85 % protein), 4 g con oil (4 % fat), 3.5 g minerals (3.5% minerals), 1 g vitamins mixture (1 % vitamins), 5 g cellulose (5 % fiber), choline chloride 0.25 and corn starch up to 100 g according to Reeves *et al*. [13]. The salt mixture used in this experiment was made according to Hegsted *et al*. [14] and the vitamin mixture according to Horwitz [15].

Biological Investigation: After this week rats were divided into 5 Groups (6 rats each) as follow, the first group was fed on basal diet as a negative control, while other groups were fed on basal diets containing 100 mg of prednisone as a glucocorticoid/ kg diet (GCD) to induce bone loss. Of them, the positive control group was kept feeding on GCD alone, while others were fed on GCD containing 30, 50 and 70 g of flaxseed powder/kg diet.

The experiment period lasted for (8weeks) during which the quantities of diet, which were consumed and/or wasted, were recorded every day. Also, the rat's weight was recorded weekly. At the end of the experiment, rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from the hepatic portal vein and left for 10 minutes to clot at room temperature, then centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated and transferred in to dry clean Eppendorf tubes and kept frozen at - 20°C until analysis.

The liver, kidney, heart and spleen were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution (0.9%), dried by filter paper and weighted to calculate organs weight/body weight %.

Biological Evaluation: At the end of the experiment feed intake, body weight gain and organs weight as a percent of total body weight were determined According to Chapman *et al.* [16].

Measurement of Bone Mineral Density and Bone Mineral Concentration: Bone mineral density (BMD) and bone mineral concentration (BMC) were measured in National Research Center, Osteoporosis Unit, by dual energy x-ray absorptiometry (DEXA) as a classification of the World Health Organization, 1994 according to Lochmüller *et al.* [17].

Determination of Calcium and Phosphorus in Femur Bones: The contents of both calcium and phosphorus in left femur bones were determined by atomic absorption spectrophotometer (Model: AvantaÓ, Australia) according to Horwitz [15].

Biochemical Analysis: The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. calcium according to Baginski *et al.* [18]. Phosphorus [19] and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel [20].

Histopathological Examination: Right femur bone of each sacrificed rat was taken and immersed in a 10% buffered neutral formalin solution till histopathologically examined according to Drury and Wallington [21].

Statistical Analysis of Data: Statistical analyses were performed by using a computer program statistical package for social science (SPSS), using Duncan Multiple Range test (One-way ANOVA test) according to Armitage *et al*. [22]. The results were expressed as mean ± SD.

RESULTS

Effect of Flax Seed as Anti-Osteoporosis Agent on Dietary Parameters in Female Rats Dieted with Glucocorticoid (GC): As shown in the Table (1) concerning the (+ve) control group, it was observed that feed intake decreased significantly $P < 0.05$ (6.98 ± 0.53 g/d/rat) in comparison to the (-ve) control group (8.58 ±0.98 g/d/rat). In respect to rats which fed on GC -containing diet supplemented with 5 % Flaxseed showed no significant difference $p>0.05$ in feed intake as compared to the (-ve) control group (7.00±0.52 and 8.58 ±0.98 g/d/rat, respectively). All other treated groups significantly decreased $P>0.05$ in feed intake (FI) when compared with the (+ ve) control group.

It could be noticed that the (+ve) control group decreased significantly $P>0.05$ in body weight gain (BWG) % (48.38±7.93) in comparison to the (-ve) control group (56.09±4.27). No significant differences $P>0.05$ in BWG% occurred among the (+ve) control group and all other groups. Concerning FER, the (+ve) control group decreased significantly $P>0.05$ (0.122±0.022) in comparison to the (-ve) control group (0.154±0.033). No significant differences $P>0.05$ in FER occurred among the (-ve) control group and all treated groups except rats fed on GC -containing diet supplemented with 7% Flaxseed.

At the final, it could be observed that the best result for feed intake, BWG % and FER regarding treated groups was recorded by group received GC -containing diet supplemented with 5% Flax seed (7.00±0.52 g/d/rat), (49.77±3.81) and (0.125±0.011) respectively.

Table 1: Effect of flax seed as anti-osteoporosis agent on dietary parameters in female rats dieted with GC

	Parameters		
Groups	FI (g/d/rat)	BWG %	FER
(-ve) Control	8.58 ±0.98^{a}	56.09±4.27^{a}	0.154±0.033^{a}
(+ve) Control	6.98±0.53^{b}	48.38±7.93^{c}	0.122±0.022^{b}
3% Flaxseed	6.95±0.48^{b}	49.45±3.28^{abc}	0.124±0.010^{ab}
5% Flaxseed	7.00±0.52^{ab}	49.77±3.81^{abc}	0.125±0.011^{ab}
7% Flaxseed	6.41±1.21^{b}	49.04±3.64^{bc}	0.122±0.010^{b}

Different superscript letters in the column denote significant differences (P<0.05)

Table 2: Effect of Flax seed as anti-osteoporosis agent on relative organs weight in female rats dieted with GC

	Parameters			
Groups	Liver	Kidney	Heart	Spleen
(-ve) Control	3.99±0.74^{e}	1.39±0.43^{c}	0.97±0.39^{d}	0.99±0.43^{d}
(+ve) Control	6.97±1.13^{a}	3.99±0.64^{a}	2.52±0.69^{a}	3.80±0.86^{a}
3% Flaxseed	4.43±0.72^{de}	2.57±0.11^{b}	1.18±0.21^{c}	2.15±0.21^{c}
5% Flaxseed	6.40±0.19^{abc}	3.42±0.20^{ab}	2.07±0.18^{ab}	3.01±0.16^{abc}
7% Flaxseed	5.99±0.94^{abc}	3.48±1.08^{ab}	1.85±0.95^{abc}	2.97±1.01^{bc}

Different superscript letters in the column denote significant differences (P<0.05)

Effect of Flax Seed as Anti-osteoporosis Agent on Relative Organ Weight in Female Rats Dieted with GC: As shown in the table (2) It could be noticed that, liver weight/body weight % of the (+ve) control group showed a significant increase P?0.05than the (-ve) control group (6.97±1.13 and 3.99±0.74, respectively).

Non-significant difference P>0.05 in liver weight/body weight % among the (+ve) control group and the groups treated with 5 and 7% Flax seed (6.97±1.13, 6.40±0.19 and 5.99±0.94 respectively). On the other hand, there were non- significant difference P< 0.05 among the (–ve) control group and the group treated with 3% Flaxseed (3.99±0.74 and 5.12±0.99 respectively) which recorded the best results. Regarding the mean value of kidney weight/body weight %, it could be noticed that the (+ve) control group showed a significant increase P<0.05 than the (-ve) control group (3.99±0.64 and 1.39±0.43, respectively). All treated groups showed no significant difference P<0.05 in kidney weight/body weight % as compared to the (+ve) control group except the group treated with 3% Flaxseed which recorded a significant decrease.

Concerning the mean value of heart weight/body weight %, it could be noticed that the (+ve) control group showed a significant increase P<0.05 than the (-ve) control group (2.52±0.69 and 0.97±0.39, respectively). All treated groups showed no significant difference P <0.05 in heart weight/body weight % as compared to the (+ ve) control group except rats fed on GC -containing diet supplemented with 3% Flaxseed. Regarding the mean value of spleen weight/body weight %, it could be noticed that the (+ve) control group showed a significant increase P<0.05 than the (-ve) control group (3.80±0.86 and 0.99±0.43, respectively). All treated groups showed a significant decrease in spleen weight/body weight % as compared to the (+ ve) control group except rats fed on GC -containing diet supplemented with 5% Flaxseed. The best result was recorded for rats fed on GC - diet supplemented with 3% Flaxseed (2.15±0.21).

Effect of Flax Seed as Anti-Osteoporosis Agent on Serum Transaminase in Female Rats Dieted with GC: As shown in the Table (3), the mean values ± SD of AST and ALT level in the serum of (+ve) control group increased significantly P< 0.05, compared to the (-ve) control group. All treated groups showed a significant decrease P<0.05 in serum AST and ALT levels as compared to the (+ ve) control group. No significant differences P<0.05 in serum AST and ALT levels occurred among all treated groups. All treated groups showed significant decrease P<0.05 in AST and ALT levels in serum as compared with the (+ ve) control group. The best result was recorded for rats fed on GC - diet supplemented with 7% Flaxseed. (221.50±33.46and 49.33±12.09).

Effect of Some Flax Seed as Anti-Osteoporosis Agent on Serum Calcium and Phosphorus in Female Rats Dieted with GC: As shown in the Table (4), the mean values ± SD of serum calcium and phosphorus levels in the serum of (+ve) control group was decreased significantly P< 0.05 compared to (- ve) control group. All treated group showed significant increase P< 0.05 in serum calcium and

Table 3: Effect of some phytoestrogens sources as anti-osteoporosis agents on Serum transaminases in female rats dieted with GC

	Parameters	
Groups	AST(U/L)	ALT(U/L)
(-ve) Control	122.50±9.63^{c}	29.67±1.97^{c}
(+ve) Control	403.17±153.30^{a}	92.83±33.32^{a}
3% Flaxseed	229.83±58.21^{b}	60.33±13.34^{b}
5% Flaxseed	239.00±52.03^{b}	54.00±7.18^{b}
7% Flaxseed	221.50±33.46^{b}	49.33±12.09^{b}

Different superscript letters in the column denote significant differences (P<0.05)

Table 4: Effect of some phytoestrogens sources as anti-osteoporosis agents on serum calcium and phosphorus in female rats dieted with GC

	Parameters	
Groups	Ca (mg/dl)	P (mg/dl)
(-ve) Control	12.483±0.71^{a}	7.733±0.24^{a}
(+ve) Control	8.400±0.52^{d}	5.600±0.28^{c}
3% Flaxseed	9.983±0.75^{bc}	7.423±1.29^{ab}
5% Flaxseed	10.050±0.27^{bc}	7.300±0.14^{ab}
7% Flaxseed	10.375±0.18^{b}	7.300±0.09^{ab}

Different superscript letters in the column denote significant differences (P<0.05)

Table 5: Effect of some phytoestrogens sources as anti-osteoporosis agents on bone health-related parameters in female rats dieted with GC

	Parameters			
Groups	BMC (g)	BMD (g/cm^2)	FCa (mg/g dry weight)	FP (mg/g dry weight)
(-ve) Control	0.508±0.001^{a}	0.128±0.002^{a}	5.866±0.10^{a}	2.95±0.05^{a}
(+ve) Control	0.203±0.001^{e}	0.092±0.001^{c}	3.916±0.08^{d}	2.12±0.12^{e}
3% Flaxseed	0.244±0.002^{d}	0.093±0.0005^{c}	4.850±0.05^{c}	2.65±0.05^{c}
5% Flaxseed	0.288±0.001^{c}	0.119±0.01^{b}	5.450±0.055^{b}	2.55±0.05^{d}
7% Flaxseed	0.452±0.001^{b}	0.121±0.0005^{b}	5.433±0.08^{b}	2.85±0.05^{b}

Different superscript letters in the column denote significant differences (P<0.05)

phosphorus levels as compared to the (+ ve) control group. Concerning to calcium levels There was no significant difference P < 0.05 among all treated groups when compared to each other. No significant differences P<0.05 in phosphorus levels occurred among all treated groups and (- ve) control group.

Effect of Flax Seed as Anti-Osteoporosis Agents on Bone Health-Related Parameters in Female Rats Dieted with GC: As shown in Table (5), It could be noticed that (+ve) control group recorded a significant decrease in bone mineral density (BMD), bone mineral concentration (BMC), femur calcium (FCa) and femur phosphorus (FP) compared with (-ve) control group. Concerning BMC all treated groups recorded a significant increase as compared to (+ve) control group, while they could not reach to the normal value recorded by the (-ve) control group.

The best result for BMC among the treated groups was for the group treated with 7% Flaxseed (0.452±0.0005). Except for the group treated with 3% Flaxseed, all treated groups recorded a significant increase in BMD as compared to (+ve) control group although they could not reach the normal value recorded by the (-ve) control group. The best result for BMD among the treated groups was for the group treated with 7 % Flaxseed (0.121±0.0005).

Concerning FCa all treated groups recorded a significant increase as compared to (+ve) control group, while they could not reach the normal value recorded by the (-ve) control group. No significant difference P<0.05 in FCa occurred between groups treated with 5 and 7 % Flaxseed (5.450±0.055 and 5.433±0.08 respectively). The best result for FCa among the treated groups was for the groups treated with 5 and 7% Flaxseed. All treated groups recorded significant increase in FP as compared to (+ve) control group, while they could not reach to the normal value recorded by (-ve) control group. The best result for FP among the treated groups was for the group treated with 7% Flaxseed.

Histopathological examination of bone:

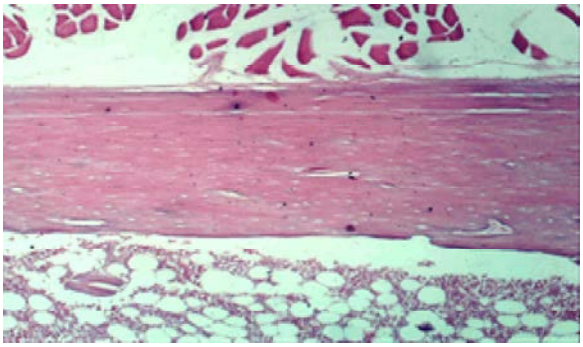

Fig. 1: Photomicrograph of the bone of rat from group 1 showing no histopathological changes. note normal bone cortex (H & E X 100)

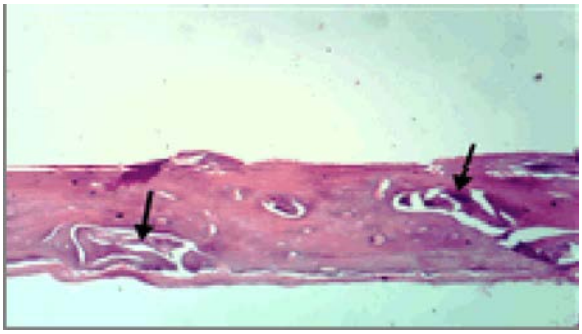

Fig. 2: Photomicrograph of the bone of rat from group 2 showing thin cortical bone with the presence of focal necrosis and cracks (H & E X 100)

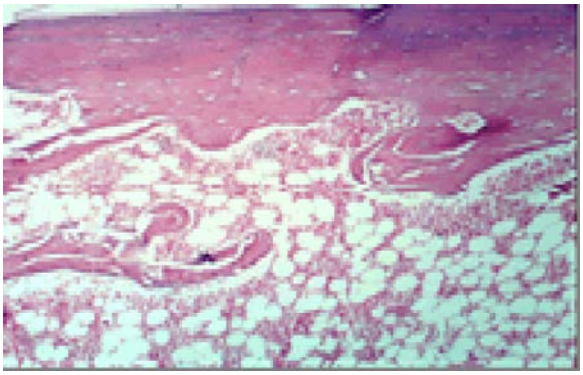

Fig. 3: Photomicrograph of the bone of rat from group 3 showing no histopathological changes. Note normal cortical bone (H & E X 100)

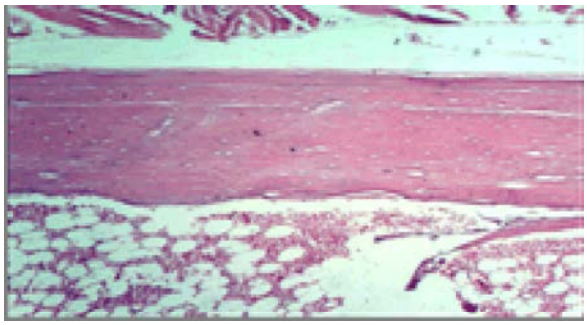

Fig. 4: Photomicrograph of the bone of rat from group 4 showing no histopathological changes. (H & EX 100)

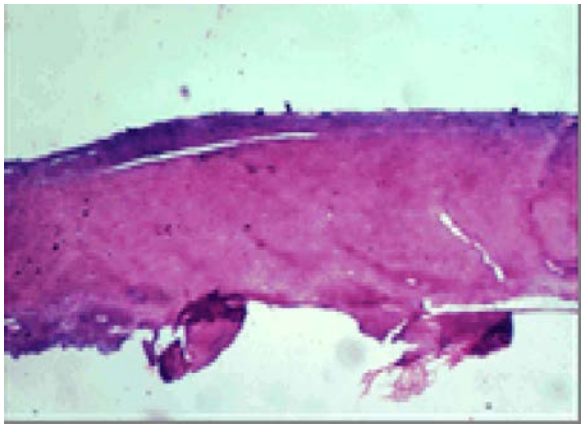

Fig. 5: Photomicrograph of the bone of rat from group 5 showing no histopathological changes (H & E X 100)

DISCUSSION

Feeding on a diet contain GC, in the present study, resulted in a significant decrease in feed intake (FI), body weight gain (BWG) and hence feed efficiency ratio (FER) compared with a healthy control group fed on basal diet. This is in agreement with Liu *et al.* [23] who found that GC treatment diminished craving and body weight of the rodents. It was demonstrated that glucocorticoids, particularly at high dosages, incited obvious glycolipid metabolic aggravations and hyperinsulinemia [24]. On the other hand Dodin *et al*. [25] showed that flaxseed consolidation into the eating routine had no clinically huge impact on weight gain and BMI.

These results are in agreement with Al-Anazi *et al.* [26] who revealed the significant job of phytoestrogen in treatment and keep up relative organs. The elevated effect of glucocorticoid –containing diet on serum transaminases as the most specific markers of liver injury, as indicated in the present study, are in line with Xu *et al.* [27], they revealed that sedate initiated liver damage is for the most part brought about by utilization of antibacterial and glucocorticoids and comprises around one fifth of hospitalized patients with ALT. Drake *et al.* [28] additionally uncovered that pre-birth glucocorticoid over presentation in rodents expands hepatic lipid aggregation with steatosis. Bhatia *et al.* [29] recommended that the assurance managed by flaxseed might be ascribed to its constituents who incorporate omega-3 fundamental unsaturated fats and phytoestrogenic lignans which seem to assume a significant job in free radical searching.

Corticosteroids assume a significant job in decreasing calcium assimilation in the small digestive system, a capacity that relies upon nutrient D [30] they likewise follow up on kidney cells to diminish renal cylindrical calcium reabsorption. Also Canalis *et al.* [31] recorded that; GCs effectively affect calcium digestion, through diminished gastrointestinal retention of calcium, promoting renal calcium misfortune.

The impact of GC - containing diet on serum dimensions of both calcium and phosphorus, recorded in the present investigation, is in concordance with De Nijs [32] who expressed that bone misfortune is a standout amongst the most significant symptoms of glucocorticoid use, even in low dosages. The primary impact of glucocorticoids on bone is a hindrance to osteoblast work, prompting a lessening in bone development. Moreover, nongenomic impacts (Intervened by glucocorticoid associations with organic layers, either through authoritative to film receptors or by physicochemical collaborations) may have a job in the pathogenesis of glucocorticoid-actuated osteoporosis (GIOP). What's more, optional hyperparathyroidism initiated by the negative parity of calcium because of restraint of retention and increment of discharge is a significant fundamental instrument of GIOP [33]. Then again, hydrocortisone can do somewhat hindering the intestinal retention of phosphate [34].

Phytoestrogens sustaining, in the present investigation, prompted a critical increment in serum calcium and phosphorus. For the most part, the expansion in the serum dimensions of the two minerals, particularly Ca, could be because of an improvement in stomach related procedures and, in this manner, to a superior intestinal retention just as a higher accessibility of the supplements in the feeds on account of the bio practical flaxseed. In this respect Kurzer and Xia [35] incorporated potential systems of activity to clarify the beneficial impact of phytoestrogens on bone misfortune. These systems incorporate avoiding urinary calcium misfortune. Bone mineral density (BMD) is known to diminish with

the drawn-out utilization of oral glucocorticoids and there is proof that treatment with 5 mg or higher portion of prednisone or proportionate builds the danger of crack by 75 % following 12 weeks [36].

The present study results are in harmony with De Nijs [32] who expressed that bone misfortune is a standout amongst the most significant reactions of glucocorticoid use, even in low dosages. The fundamental impact of glucocorticoids on bone is the restraint of osteoblast work, prompting an abatement in the bone arrangement. Likewise, non-genomic impacts (Mediated by glucocorticoid interactions with biological membranes, either through binding to membrane receptors or by physicochemical interactions) may have a job in the pathogenesis of glucocorticoid-initiated osteoporosis (GIOP). What's more, auxiliary hyperparathyroidism instigated by negative parity of calcium because of restraint of ingestion and increment of discharge is a significant fundamental instrument of GIOP. More up to date wholesome thoughts, with a soundproof base for a constructive outcome on bone wellbeing, are showing up, including nutrient K, phytoestrogens and dietary soluble base.

Tham *et al.* [37] speculated that an eating regimen rich in isoflavones protectively affects bone. Vincent *et al.* [38] presumed that genistein has a biphasic impact, lower portions improved bone mineral instead of high dosages, on bone mineral thickness in ovariectomized rodents. Van der Linden *et al.* [39] evaluated three investigations in bone mineral thickness with phytoestrogen utilization that were led with postmenopausal ladies. Two of the examinations demonstrated an expansion in bone mineral thickness.

Kurzer and Xia [35] looked into a few different investigations that incorporate potential systems of activity to clarify the beneficial impact of phytoestrogens on bone misfortune. These instruments incorporate avoiding urinary calcium misfortune, beneficial consequences for osteoblasts and influences on the discharge of calcitonin which stifles bone resorption. Moreover, estrogen receptors have been found in osteoblasts, which may cause an adjustment in some protein generation.

Jones *et al.* [9] detailed the significant job of phytoestrogen in the counteractive action of osteoporosis. In this regard Wang *et al.*[40] discovered that low dosages of phytoestrogens joined with calcium and nutrient D may expand BMD and improve the mechanical quality of bone.

Salari *et al.* [41] outlined a few examinations as a major aspect of a meta-investigation of accessible information. They recommended that dietary admission of forerunners for the ω-6 and ω-3 pathways are fundamental and adjust film structure.

The result of the present study agrees with Arjmandi *et al*. [42] who reported that; postmenopausal women consuming flaxseeds (38 g/d; 6 wk.) showed a slightly lower bone resorption marker (Tartrate-resistant acid phosphatase) and no changes in other bone-formation indicators or anabolic bone agents (Alkaline phosphatase, Insulin-like growth factor binding protein 3 (IGFBP-1), Insulin-like growth factor binding protein 3 (IGFBP-3).

Then again can't help contradicting a few examinations led in postmenopausal ladies and uncovered that flaxseed utilization (25-45 g/d) for present moment (3- to 4-mo) or long-term (12-mo) periods does not essentially impact BMD, BMC, or bone turnover [25].

In contrast, the result agrees with Sacco *et al*. [43] who announced that supplementation of flaxseeds, alone or in blend with low-portion estrogen, likewise positively changed lipid profiles in the vertebrae and tibia by expanding ω-3 and diminishing ω-6 PUFAs, which is accepted to be useful for bone arrangement by expanding calming and repressing proinflammatory eicosanoid generation. Comparative results were seen in OVX rodents encouraged flaxseeds alone or in blend with ultra– low-portion estrogen. In spite of the fact that the bone-defensive impact was not seen in ultra-low portion estrogen bunch alone, when joined with flaxseed bolstering, more elevated amounts of ω-3 PUFAs and lower dimensions of ω-6 PUFAs in the vertebrae and tibia were watched, which was decidedly connected with higher BMD, BMC and bone quality of these skeletal destinations [44].

Maíra *et al*. [45] announced that an eating routine containing flaxseed flour had practical properties and added to bone mineral density and femur resistance at 180 d.

Flaxseed flour contains high ALA concentrations [46]. With respect to impacts, ALA is changed over into eicosapentaenoic and docosahexaenoic acids and these unsaturated fats straightforwardly follow up on bone marrow–derived macrophages and lessening osteoclastogenesis, osteoclast development and bone resorption by receptor activator of atomic factor κ beta ligand (RANKL) down-guideline. For osteoblasts, ALA protect bone mass by expanding articulation of key

interpretation factors, for example, osteocalcin, which improve preosteoblasts' separation into develop osteoblasts and bone formation. ALA produces a contrary effect: It stimulates osteoclastogenesis and decreases osteoblastogenesis [47-49]. Other than ALA, flaxseed flour contains a high calcium amount (236 mg/100 g of seed), ALA expands calcium retention in the gut, advancing mineral deposition in bones [46, 50]. Moreover, vegetal protein (As the present in flaxseed flour) may lessen natural acids and metabolic acidosis related with bone demineralization, adding to bone tissue solidness and obstruction [46, 51]. El-Saeed *et al.* [52] proved the effective impact of n-3 fatty acids in the form of flaxseed on improving skeletal formation and avoiding osteoporosis.

CONCLUSIONS

According to the current findings, it could be concluded that dietary supplementation with flaxseeds is recommended to prevent glucocorticoid-induced bone loss. The co-supplementation was more potent in elevating BMC and hence BMD due to the synergistic effect. Some of the associated side effects were also diminished.

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