The effect of supplementation with flaxseed and its extract on bone health

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Abstract: Background: Osteoporosis is a major health problem in postmenopausal women due to a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling. It is necessary to develop "natural" substance acts as alternative to traditional hormone replacement therapy with less undesirable side effects such as phytoestrogens compounds. Lignan-flaxseed compound is a type of phytoestrogens exerts many beneficial effects on human health. **Objective:** This work has been carried to investigate whether supplementation with flaxseed or its lignan extract will improve bone health in experimental animals (aged rats). **Methods:** Forty eight 13- month-old Western strain female rats were divided equally into 8 treatment groups: group 1 fed basal diet BD (control group), group 2 fed BD + orally dosed 17α ethinyl estradiol (reference group), groups 3& 4 fed BD supplemented with 5% and 10% flaxseed respectively, groups 5 & 6 fed BD+ orally dosed 0.05 g and 0.1g/day/rat lignan extract respectively and groups 7&8 fed BD supplemented with 20%and 40% flat bread (supplemented with 25% flaxseed) respectively. The experimental period continued 8 weeks. Serum total Ca, Mg, P, Alk. phosphatase, β estradiol and intact parathyroid hormone were estimated. Also, Urine Ca, P and creatinine were estimated. Bone properties and chemistry were assayed. **Results:** Our results show that the supplementation with flaxseed is more effective than lignan extract supplementation on mineral absorption especially serum Ca. There were decreasing in calcium and phosphorus excretion in urine when the aged rats subjected to all treatments. Treatment with flaxseed and its lignan extract decreased both of serum alkaline phosphatase (bone formation marker) and parathyroid hormone (bone resorption marker) levels but did not affected on serum β-estradiol levels. Moreover, treatment with flaxseed or its lignan extract dose-dependently increase deposition of Ca, P and Mg in bone of groups 4,6,8 which were translated into an increment in femur length, breaking force and bone mineral density (BMD). **Conclusion:** It could be concluded that daily consumption of flaxseed can prevent bone loss due to estrogen deficient. The efficacy of such dietary intervention may be act as a natural alternative to traditional hormone replacement therapy among postmenopausal women.

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1. Introduction

Osteoporosis is a major health problem in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling **(Nielsen *et al.*, 2004)**. The increased bone remodeling is associated with both decreased bone mineral density (BMD) and increased risk of fracture **(Garnero *et al.*, 2000).** Together with BMD, bone turnover markers (BTMs) have been considered to be biomarkers for fracture risk **(Miller *et al.*, 2005)**. BTMs can be used for the diagnosis and evaluation of therapy effects on osteoporosis **(Nishizawa *et al.*, 2005),** and include bone resorption markers and bone formation markers. BTMs change earlier and to a larger extent than changes in BMD or risk of fracture. The decrease in BTMs in the early stages of treatment may reflect a reduction in the long-term risk of fracture **(Eastell *et al.*, 2003).** Because hypoestrogenemia after menopause is an important cause of osteoporosis, hormone replacement therapy (HRT) was used to be a popular regime for prevention and treatment of postmenopausal osteoporosis **(Stevenson, 2005).** However, HRT is associated with a higher risk of hormone-related cancer **(Anderson *et al.*, 2003)** and other unfavorable adverse events **(Morabito *et al.*, 2002)**.

Although traditional therapeutic agents that stimulated bone formation (e.g., sodium fluoride, growth hormone, and anabolic steroids and antiresorptive agents (e.g., calcitonin and bisphosphonates) may prevent further bone loss in established osteoporosis, their costs are too high to benefit a large population in the developing or even the developed countries for prevention and treatment of osteoporosis. Consequently, it is necessary to develop "natural" products or synthetic substance with less undesirable side effects that can substitute or reduce the need for drugs used currently **(Kessel, 1998).** Dietary phytoestrogens are compounds that are estrogen like in structure and can elicit both weak estrogenic and antiestrogenic activities **(Adlercreutz, 2002).** They include the lignans, found in most plant foods but in the highest concentrations in flaxseed

(Thompson, 2003), and the isoflavones, which are abundant in soy and soy products **(Turner *et al.*, 2003).** Flaxseed is increasingly used as an ingredient in food products **(Oomah, 2001),** because of its high alpha-linolenic acid and dietary fiber content, but recently also because of its secondary metabolites. Important secondary metabolites are lignans, which are present in flaxseed in a higher concentration than in other edible sources **(Milder *et al.*, 2005).**The main lignan in flaxseed is secoisolariciresinol diglucoside (SDG), which is present in defatted flaxseed flour in concentrations up to 3% (w/w) **(Johnsson *et al.*, 2000)**. It is metabolized into the more biologically active mammalian lignansenterodiol (END) and enterolactone (ENL) by microbiota in the colon **(Jacobs *et al*., 1999).**

Other lignans present in flaxseed are matairesinol (MAT), isolariciresinol (isoLARI), pinoresinol (PINO), and lariciresinol (LARI). Other phenolic compounds reported in flaxseed, which might contribute to the health effects ascribed to flaxseeds, are hydroxycinnamic acids like p-coumaric acid ,ferulic acid, sinapic acid, caffeic acid and their glucosides, and the flavonoids herbacetin diglucoside (HDG) and kaempferol diglucoside (KDG) .Lignans are reported to exhibit protective effects against hormone-related types of cancer like breast cancer **(Boccardo *et al.*, 2004)** and against non-hormone related colon cancer **(Sung *et al.*, 1998).** Furthermore, they lower the risk of cardiovascular diseases **(Lucas *et al.*, 2004)** and obesity **(Park and Velasquez, 2012).** Recently, attention also has focused on the possible role of lignans, secoisolariciresinol diglycoside from flaxseed and isotaxiresinol from Taxuxyunnanens is prevented bone loss in postmenopausal women or ovariectomized (OVX) model, respectively **(Kim *et al*., 2002) (Yin, 2006)**. The effect of lignans on postmenopausal BMD is uncertain. Others have shown that lignan-rich flaxseed may induce weak beneficial effects **(Arjmandi, 2001)** As the result of controversial data, the present study was aimed to evaluate the influence of flaxseed and its extract (lignan complex) supplementation on bone health in estrogen deficient rats.Orally dosed 17α-ethinylestradiol (E2) was used as a reference compound for estrogenic activity on bone.

2. Materials & Methods

Materials:

Flaxseeds (*Linum usitatissimum*) Sakha 8 was obtained from Field Crops Research Institute, Fiber department. Flaxseeds were underwent cold mechanical pressing to extract its oil content in National Research Center. So, flaxseed samples used in this experiment were partially defatted (the estimated oil content of the partially defatted flaxseeds was 16 %).

Preparation of lignan extract from flaxseed

Flax lignan was prepared according to method of **Karin *et al.* (2007)** with some modification as follows: flaxseeds were ground in a coffee grinder to obtain a fine powder which defatted by n-hexane (1:6 w/v) for 12 h at 25 ċ . Defatted flaxseeds were air dried for 12 h. The lignan macromolecule was extracted from the defatted flaxseeds by a three-step sequential extraction with 63% (v/v) aq. ethanol. In the first step, 9 ml of aq. Ethanol per gram of defatted flaxseeds was used, in the second step 3.6 ml/g and in the last step 2.3 ml/g. The first two extractions were performed for 4 hrs at room temperature while stirring, the last extraction was performed overnight. The extract was filtered and then was concentrated at
50 °C by a rotary evaporator at 90 rpm. Then, the extract was lyophilized.

Quantification of polyphenolic compounds

Polyphenolic compounds included **secoisolariciresinol** in flaxseed was analyzed by HPLC. A high performance liquid chromatography system equipped with a variable wave length detector (Agilant, Germany) 1100, autosampler, quaternary pump degasser and column compartment. Analyses were performed with a C_{18}reverse phase packed stainless-steel column (Zorbax ODS 5 μm 4.6 ×250 mm). The chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by (**Schieber *et al.*, 2001**).

Preparation of bread

Baking Test

Experimental bread making was done according to the food technology research Institute, baking and pasta department procedure as described below. Control flat bread mad using wheat bran at 50% replacement level of the flour. The dough was made from flour, compressed yeast 1.5 %, salt, 1.0% sugar and water 50%. Dough was mixed at 30 °C then covered and kept at 25 ± 2°C for 10 min. The dough was divided into small pieces (130-150g) rounded and sheeted then placed on board for final proofing. Finally, it was baked at 280**°C.** Flaxseed bread supplemented formulas were prepared as shown in Table (1).

Sensory Evaluation

The effect of the replacement of flour and wheat bran with flaxseed flour showed by sensory evaluation. Sensory evaluation of the baked loaves quality characteristics was carried out following cooling to room temperature for 2 h. Sensory evaluation was performed by a trained panel of 10 judges from Food Tech. Res. Inst. Loaves were randomly assigned to each panelist. The panelists

were asked to evaluate each loaf for appearance, crumb texture, crumb grain, crust color, taste, odor and overall acceptability. A 5 point scale was used where 5 excellent and 1 extremely unsatisfactory. Samples with scores of <2 were regarded as unacceptable for sale and with scores of<1.5unacceptable for human consumption. (***Sourki et al., 2010)***

Animals and treatments

Forty eight 13- month-old Western strain female rats with body weight 250 ± 5 g (model of postmenopausal women) were housed in animal house of Ophthalmology Research Institute at12-h light/ dark cycle in a temperature- and humidity-controlled room. The animals were allowed free access to feed on basal diet (BD) that was based on AIN -93 G formulation with some modification (**Reeves *et al.*,1993**) and water *ad libitum* in the acclimatization period (one week). After this period, animals were divided equally into 8 treatment groups of equal mean weights (6 rats/ group).

- Group (1): fed on BD (control group).
- Group (2): fed on BD +orally dosed 17α ethinylestradiol (E2) (25 µg /Kg body weight/day) through a stomach tube.
- Groups (3) and (4): fed on BD supplemented with 5 and 10 g ground flaxseed per 100g BD respectively.
- Group (5) and group (6): fed on BD+ orally dosed lignan extract at concentration 0.05 and 0.1g/1ml dist.H_2O/day/rat respectively through a stomach tube. The lignan extract concentration in groups 5and 6 was estimated to be equivalent to those taken in the respective flaxseed diets in groups 3and 4. This estimation was done according to the yield of lignan extract from defatted ground flaxseed (450 g flaxseed yielded approximately 30 g lignan extract) and the mean daily food intake was approximately 15 g.
- Group (7) and (8): fed on BD supplemented with 20 and 40 g bread (supplemented with 25% flaxseed) per 100g BD respectively to be equivalent to those taken in the respective flaxseed diets in groups 3 and 4.

The BD supplemented with flaxseed or bread (supplemented 25% flaxseed) was corrected for protein, fat and fiber contributed by flaxseed so that the energy value of the diets was the same (Table 2). The experimental period continued 8 weeks. During the experiment, water was allowed *ad-libitum*.

The body weight was recorded weekly and the food intake was recorded each two days. Urine sample was collected from the rat that was housed individually for 24 h in metabolic cages without providing food 1 day before euthanizing the animals and acidified with 2 ml 1 mol/L HCL. After laparotomy using anesthetized with diethyl ether, blood sample was collected via abdominal aorta puncture, serum was then prepared by centrifugation of the collected blood (2000 rpm for 20 min). Urine and serum samples were then stored at −20 °C for biochemical determinations. Right and left Femurs and lumbar vertebra (LV1-LV6) were dissected, cleaned, freed from the surrounding tissues and filled in physiological saline and stored at−20 °C.

Table (1) Flaxseed Flat bread Formulas

Treatments	Flour 72% Ext. (g)	Wheat bran (g)	Defatted Flaxseed (g)
Treatment 1 (control)	50	50	-
Treatment2	75	12.5	12.5
Treatment 3	50	25	25
Treatment 4	75	-	25
Treatment 5	50	-	50

Table (2): composition of experimental diets

Ingredients (g/100g diet)	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**
Casein(14%protein)	20	20	19.7	16.7	20	20	14.7	9.4
Corn oil	4	4	3.2	2.4	4	4	3.2	2.5
Cellulose	5	5	4.7	4.3	5	5	4.6	4.2
Mineral mix,AIN 93	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix,AIN 93	1	1	1	1	1	1	1	1
Sucrose	10	10	10	10	10	10	10	10
L-Cystine	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Corn starch	56	56	57.5	61.7	56	56	62.6	68.9

Assay of serum and urine chemistry

Serum and urine total calcium and phosphorus were estimated calorimetrically by the method given by **Weatherburn *et al.* (1982)** as cited in Linear Chemicals. Serum magnesium was estimated according to the method of **Bohuon *et al.* (1962)** as cited in Quimica Clinica Aplicada. Serum alkaline phosphatase was measured by standard colorimetric methods using commercial kits according to method of **Burtis and Ashwood**

(1986).Urinary creatinine was quantified kinetically according to the method of **Labbe *et al.* (1996)** as cited in Biosystem.Serum β-estradiol (E2) was estimated by a competitive enzyme linked immune-sorbent assay (ELISA) method according to the manufacture's instructions (DRG International Inc., USA). Serum intact parathyroid hormone (iPTH) was estimated by a competitive enzyme linked immune sorbent assay (ELISA) method according to the manufacture's instructions (DRG International Inc., USA).

Assay of ash and mineral contents in bone

The left and right femurs were ashed for 8hrs at 750ċ .The ash weights were determined, then samples were suitably dissolved and diluted with 10% HCL to estimate Ca, Mg and P by Flame Atomic absorption spectrophotometer, AAS (Model3300, Perkin-Elmer, Beaconsfield, UK) by wet digestion to procedure of the ***AOAC (1990)***

Assay of physical properties,BMCand BMD in bone

The length and the thickness (diameter) of two femurs were determined by Venier Caliper.The measurement of bone mineral content (BMC) and bone mineral density (BMD) of right femur and lumbar vertebra was done by Dual-energy X-ray absorptiometry (DXA), Norland-XR-46 scanner,USA. The shearing force required to break the femurs was measured using Cometech, B type, Taiwan. Where three points test were performed with this machine operated at a crosshead speed of 100mm min $^{-1}$. The shear force (breaking force) needed to cut the sample with a flat ended probe (2.5mm thickness) was registered. All measurements were performed at ambient temperature 20 ċ. **(Boarne, 2003)**

Statistical Analysis

Data were presented as mean ±S.E. and analyzed by one-way analysis of variance (ANOVA) and Duncan's test ($P \leq 0.05$) were used to establish the significance of differences. The result was performed using the SPSS software version 10 windows program.

3. Results and Discussion

Phenolic fractions of flaxseed extract using HPLC (ppm)

As shown in Table (3) the data illustrated the phenolic compounds of defatted flax seed extract contains secoisolariciresinol, catechol, protocatchic acid, Chlorogenic acid, caffeic acid, caffeine acid and ferulic acid. Catechol showed the highest amount of phenolic compounds. The amount of Secoisolariciresinol is 991.7 ppm meanwhile, the concentration of ferulic acid; P- coumaric is 95.31 and 17.47 ppm respectively. The obtained results are in good agreement with **Herchi *et al.* (2011)**

Table (3) Phenolic fractions of defatted flaxseed extract using HPLC (ppm)

Phenolic compounds	ppm
Secoisolariciresinol	991.70
Catechol	1876.62
Protocatchoic	46.82
Chlorogenic	5.20
Caffeic	27.75
Ferulic	95.31
Caffien	75.58
p- Coumaric	17.47
Chrisin	35.26

Sensory Evaluation of flat bread

Based on overall acceptance. It could be seen that treatment 4 (75%flour+25% flaxseed), this treatment used in making flat bread up to 25% flaxseed without affecting their studied sensory characteristic. ***Hussain et al (2011)*** concluded that addition of up to 12% full fat and partially defatted flaxseed flour in the pan breads does not negatively affect the sensory scores. The replacement of roasted partially defatted flaxseed flours up to a level of 16% supplemented in whole wheat flour was found acceptable regarding the sensory attributes of chapattis (unleavened flat breads***) (Hussain et al., 2008).***

The results showed no significant differences between the bread formulas in texture in all treatments. Based on color (preferred white color) and flavor, it could be seen that flaxseed replaced flour and bran used in making flat bread up to 25% without affecting their studied sensory characteristic .When the flaxseed was replaced with 50%, the produced bread unaccepted as judged by panelists due to the dark color and flavor. The decrease in taste and aroma of pan breads by the increase in level of flaxseed flour supplementation might be due to more nutty taste ,higher fat contents***(Hussain et al .,2011). Frank and Sarah (2006)*** stated that addition of 15% flaxseed meal in bread negatively affected the volume, crust color, and crumb color of breads .The work of other researchers also supported the findings of the study as ***Koca and Anil (2007)*** *who* showed that crumb darkness increased by increasing the level of flaxseed flours levels. Significant decrease in assigning scores to all the sensory attributes at breads exceeding 25% flaxseed. In the present study, assignment of fewer score by the panelists to the crust and crumb color of (50% supplementation flaxseed) breads attributed to the darker color of flaxseed flour imparted to resultant breads as the flaxseed meal (flour) is darker than wheat flour and bran. The darker color has also been attributed to Millard reaction between reducing sugars phenolic compounds and proteins during baking process as mentioned by ***Dhringra and Jood***

(2004). According to sensory, 25% flaxseed bread supplemental (treatment 4) were chosen for biological test.

Effect of flaxseed and its lignan extract supplementation on food intake, body weight and food efficiency ratio.

As shown in Table (4), food intake was more or less similar among all treatment groups except group (8) which had the highest value and it was significantly different as compared with group (1). This increment in food intake for group (8) may be due to the increase supplementation of diet with 40% fortified bread. There was no significant difference in body weight gain between the control group 1 and groups 2, 3, 4, 5, 7 and 8 but group 6 was significantly lower than group 1 (Table 4). The decrease in body weight of Group 6 attributed to feeding on BD supplemented with high dose of lignan extract (0.1 g/day) .Consequently, the food efficiency ratio was affected by this decrement. The present result was confirmed by **Park and Velasquez (2012)** who suggested that lignan-enriched flaxseed powder had beneficial effects on reduction of body weight and fat accumulation through controlling in hormones related obesity (decrease in leptin level and increase in adiponectin).

Table (4) Effect of with flaxseed and its extract supplementation on food intake, body weight gain and food efficiency ratio

Parameters / groups	1 Control	2 E2	3 Flax 5 %	4 Flax 10 %	5 0.05 g Lig	6 0.1 g Lig	7 20 % FB	8 40 % FB
Food intake (g/d)	14.44^{b} ±0.82	15.30^{ab} ±0.44	14.58^{ab} ±1.21	14.94^{ab} ±0.66	15.00^{ab} ±0.32	14.28^{b} ±1.16	15.90^{ab} ±0.78	17.10^{a} ±0.34
Body weight gain (g)	0.31^{a} ±0.08	0.35^{a} ±0.05	0.15^{ab} ±0.06	0.24^{ab} ±0.06	0.22^{ab} ±0.03	0.02^{ab} ±0.03	0.40^{a} ±0.134	0.40^{a} ±0.134
food efficiency	0.020^{a} ±0.006	0.023^{c} ±0.003	0.011^{abc} ±0.004	0.007^{ab} ±0.001	0.013^{abc} ±0.0030	0.001^{b} ±0.0003	0.016^{a} ±0.004	0.020^{ac} ±0.006

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test. E2**:** 17α ethinylestradiol –Lig :lignan – FB : flat bread

Effect of flaxseed and its lignan extract supplementation on serum and urine biochemical analysis

As noticed in Table (5), levels serum total calcium were significantly increased in treatment groups 2,3,4 and 8 but in groups 5,6 and 7 show non-significant increment as compared with the control group 1. Although levels of serum magnesium in treatment groups supplemented with 10% flaxseed,0.1g lignan extract and 40% flax bread (groups 4,6,8 respectively) recorded the highest values but no significant difference between these groups and the control group 1.With respect to values of serum phosphorus, no significant difference among groups except group 4 that supplemented with 10% flaxseed when compared with control one.Our result showed that the supplementation with flaxseed is more effective than lignan extract supplementation on mineral absorption especially serum ca. The possible reason is that flaxseed was partially defatted but it still contained 16% oil, therefore flaxseed contained omega 3 fatty acid, another bioactive substance beside lignin compounds. This increase in ca absorption indicated an increase in the transference of calcium concomitantly with an increase in the activity of alkaline phosphatase and Ca^{2+} ATPase, the two intestinal mucosal enzymes which have a significant contribution to the regulation of intestinal transference of calcium and thus suggest its phytoestrogenic efficacy mediating these actions **(Hernandez *et al.*, 2004)**. The reduction of estrogen in postmenopausal women is possibly responsible for inhibiting the hydroxylation of 25-hydroxy vitamin D3 to 1, 25-dihydroxy vitamin D3 and reducing the intestinal transference of calcium. This result also finds support from an earlier proposed hypothesis that menopause and estrogen deficiency are associated with intestinal resistance to 1, 25-dihydroxy vitamin D3 and thereby reduce calcium absorption **(Gennari *et al.*, 1990).** The increment in ca absorption is confirmed by decreasing Ca/cr and P/cr excretion in urine when the postmenopausal rats subjected to all treatments as shown in Table (5).

As shown in Table (5), the treatment group 2 which supplemented with E_2 had the highest mean value of serum 17 β- estradiol and was significantly different from control group and group that supplemented with low dose of lignan extract (treatment 5) but no statistical differences with other groups supplemented with flaxseed. In support of our results, **Brooks *et al.* (2004)** found that supplementation the diet of postmenopausal women with 25 g ground flaxseed for 16 weeks had no effect on serum estradiol or estrone concentration. Also, they reported that supplementation with flaxseed increased the urinary excretion of 2-hydroxyestrone (less biologically active estrogen metabolite) that positively related to urinary lignan excretion. Similarly, in study of **Arjmandi *et al.* (1998)**,

flaxseed consumption had no effect on serum levels of follicle stimulating hormone and 17β- estradiol levels indicating lack of true estrogenic activity of flaxseed. It can be suggested that flaxseed or its lignan may act on bone metabolism as weakened estrogenic compounds and perhaps as antiestrogenic compounds on other estrogen sensitive tissues.

Serum alkaline phosphatase levels decreased significantly in all treatment groups as compared with control one. Concerning to levels of serum parathyroid hormone, it is noticed that treatment groups 4, 6 and 8 had the lowest mean values and all treatments decreased significantly from control group (Table 5). As demonstrated in Table 5, urine excretion of Ca/cr/day and P/cr/day showed that there were significant decrement in all treatment groups as compared with the control group .In present study, the concentration of bone biomarkers, serum alkaline phosphatase levels (bone formation marker) and serum parathyroid hormone (bone resorption marker) in control group (postmenopausal model) were higher than those in treatment groups, indicating the increase of bone turnover rate. Treatment with flaxseed and its lignan extract decreased both of serum alkaline phosphatase and parathyroid hormone levels, indicating a reduction in bone turnover.This suggests that a down regulation of bone turnover had occurred **(Huang *et al.*, 2008).** Parathyroid hormone, interlukine-1 and prostaglandin E2 which are produced, attract inflammatory cells in the proximity of osteoblasts, have adverse effects on bone formation and stimulate osteoclast bone resorption **(Fernandes *et al.*, 2003).** The present results are consistent with the finding of a study by **Babu *et al.* (2000)** who indicated that feeding whole or defatted flaxseed to weanling female rats for 56 days suppressed serum total alkalinephosphatase activity, a nonspecific marker ofbone formation .The aforementioned results can be explained by the fact that whether flaxseed or its lignans behave similarly to estrogen by suppressing both bone formation and bone resorption **(Lucas *et al.*, 2002).** In contrast to above results, **Farmer *et al.* (2007)** concluded that supplementary flax did not alter bone resorptive activity, although enhancing n-3 polyunsaturated fatty acids in the tissues.

Effect of Flaxseed and its extract supplementation on chemical, physical, mineral density and breaking force parameters in bone

As shown in Table (6), no significant differences in ash content of femurs among treatment groups except treatment group supplemented with E_2 and flaxseed 10%. The mean value of calcium content in femurs had the highest value in group supplemented flaxseed 10% followed by group supplemented with fortified bread 40% flax, then those supplemented with 0.1g lignan (groups 4, 8, 6 respectively). Concerning phosphorus in femurs in Table (6), no statistical changes between treatment groups and control group were found except group 4 (10% flax) and group 6 (0.1g lignan). Femurs magnesium content was increased significantly in all groups except treatment group supplemented E_2 as compared with control one. As illustrated in Table (6), although femur lengths showed statistical changes in all treatments but femur thickness did not show any differences as compared with control one.

As illustrated in Fig.(1), there were significant differences in breaking force (shearing force) between all treatments and control group with the highest value in treatment 6 followed by treatment 4, then treatment 8.

Fig (2) showed that femur bone mineral density (FBMD) recorded significant differences in all treatments except treatment 2 (E_2) as compared with control group with the highest value in treatment 4 followed by treatment 6, then treatment 8.While femur bone mineral content (FBMC) measurements were increased significantly in treatments 3, 4, 7 and 8 but non-significant increase occurred in treatments 2, 5 and 6.

In Fig (3) lumbar vertebrae bone mineral density (LVBMD) values were significantly higher in treatments 4, 5 and 8, while lumber vertebrae bone mineral content (LVBMC) values in treatments 3, 4 and 8 were significantly increased but the remaining treatments did not show significant changes as compared with control group.

The obtained results indicated that treatment with flaxseed or its lignan extract dose-dependently increase deposition of Ca, P and Mg in bone of groups 4, 6, 8 which were translated into an increment in femur length, breaking force and bone mineral density (BMD).These results were reflected by decreasing in serum PTH (bone resorptive marker) and urinary excretion of ca at high dose of flaxseed or its lignan (Table 5) leading to reduction in bone turnover. Our data in agreement with those of **Yin *et al.* (2006)** who reported that isotaxiresinol, the main lignan isolated from the water extract of wood of Taxusyunnanensis had a positive effect on bone metabolism by increasing BMD and BMC in total and cortical bones and decreasing bone resorption marker in ovariectomized rats.The positive effect of flaxseed or its lignan extract on bone metabolism is limited to young life in female rats may be due to the estrogenic effect induced by flax lignan occurred when natural estrogen production is low **(Ward *et al.*, 2001).** Our data supported this concept whereas the experimental rats in the present study were old (postmenopausal age) and also had low endogenous estrogen.

Kim and Ilich (2011) 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. In most animal studies (regardless of growing stages or physiologic conditions), feeding flaxseed/oil resulted in improved lipid profiles in plasma, bone, and other tissues by increasing omega-3 polyunsaturated fatty acids and decreasing omega-6polyunsaturated fatty acids levels resulting in prostaglandin biosynthesis will inhibited. Prostaglandins have been reported to stimulate osteoclastic bone resorption in organ culture **(Fernandes *et al.*, 2003).** This subsequently improved bone properties (BMD, BMC, bone strength, bone turnover) in mature and/orovariectomized animals only, not in growing animals.

Findings from this study suggest that flaxseed may provide a dietary approach for maintaining bone health after cessation of endogenous estrogen production. The efficacy of such dietary intervention may act as a natural alternative to traditional hormone replacement therapy among postmenopausal women. Therefore, the current study recommends that it could be reused the partially defatted flaxseed (cake) produced from oil extraction and incorporated it into human daily food as bread or another food product.

Table (5) Effect of Flaxseed and its extract supplementation on serum and urine biochemical analysis

Parameters / groups	1 Control	2 E2	3 Flax 5 %	4 Flax 10 %	5 0.05 g Lig	6 0.1 g Lig	7 20 % FB	8 40 % FB
S. Ca mg/dL	8.68^{b} ±0.27	12.71^{a} ±1.01	12.69^{a} ±0.93	13.55^{a} ±0.42 0.42	9.04^{b} ±0.59 0.59	8.83^{b} ±0.53 0.53	9.94^{b} ± 0.67	12.41^{a} ±0.68 0.68
S. Mg mg/dL	2.24^{ab} ±0.23	2.44^{ab} ±0.13	2.01^{b} ±0.19	2.65^{a} ±0.11	2.31^{ab} ±0.07	2.65^{a} ±0.07	2.44^{ab} ±0.09	2.53^{a} ±0.16
S. P mg/dL	5.70^{b} ±0.47	6.87^{ab} ±0.79	5.99^{b} ±0.41	7.72^{a} ±0.13	6.55^{ab} ±0.11	5.94^{b} ±0.15	6.82^{ab} ±0.07	6.27^{b} ±0.17
S. E2	24.33^{b} ±0.67	29.67^{a} ±1.33	26.67^{ab} ±1.20	25.67^{ab} ±1.21	23.00^{b} ±0.58	25.33^{ab} ±1.33	25.67^{ab} ±1.76	26.33^{ab} ±1.85
S. AlkPhos.	65.35^{a} ±0.87	42.92^{b} ±1.78	41.69^{b} ± 0.58	45.06^{b} ±0.83	42.03^{b} ±1.73	44.85^{b} ±2.68	40.89^{b} ±0.88	43.70^{b} ±0.79
S. PTH	18.25^{a} ±0.66	13.33^{b} ±0.67	11.25^{cb} ± 0.72	5.92^{d} ±0.36	10.17^{c} ±0.74	6.00^{d} ±0.63	11.93^{cb} ±0.84	6.63^{d} ±0.59
U. P/Cr/day	0.32^{a} ±0.04	0.15^{b} ±0.01	0.20^{b} ±0.02	0.15^{b} ±0.02	0.17^{b} ±0.01	0.17^{b} ±0.02	0.20^{b} ±0.03	0.18^{b} ±0.03
U.Ca/CR/day	0.13^{a} ±0.005	0.09^{b} ±0.003	0.09^{b} ±0.005	0.08^{b} ±0.003	0.09^{b} ±0.005	0.08^{b} ±0.003	0.08^{b} ±0.006	0.08^{b} ±0.008

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD test. E2**:** 17α ethinylestradiol –Lig :lignan – FB : flat bread

Table (6) Effect of Flax seed and its extract supplementation on ash weight and Ca, P, Mg content, length and thickness of rat femurs

Parameters / groups	1 Control	2 E2	3 Flax 5 %	4 Flax 10 %	5 0.05 g Lig	6 0.1g Lig	7 20 % FB	8 40 % FB
Ash weight (g)	0.50^{bc} ±0.01	0.54^{a} ±0.01	0.51^{abc} ±0.01	0.54^{a} ±0.01	0.51^{abc} ±0.02	0.50^{c} ±0.01	0.51^{abc} ±0.01	0.53^{ab} ±0.01
Ca mg/F	280.0^{c} ±10.7	295^{bc} ±8.6	311^{bc} ±6.9	351^{a} ±5.5	304^{bc} ±16.7	320^{ab} ±10.9	306^{bc} ±13.8	330^{ab} ±5.8
P mg/F	92.00^{b} ±7.9	100.83^{ab} ±4.9	98.2^{ab} ±12.4	123.6^{a} ±6.9	111.83^{ab} ±8.6	118^{a} ±1.3	109.36^{ab} ±8.8	107.06^{ab} ±8.7
Mg mg/F	3.29^{d} ±0.22	3.65^{cd} ±0.59	4.89^{ab} ±0.44	5.35^{a} ±0.16	4.95^{ab} ±0.01	5.70^{a} ±0.24	4.34^{bc} ±0.08	4.89^{ab} ±0.18
Left femur length mm	3.17^{b} ±0.06	3.43^{a} ±0.03	3.47^{a} ±0.08	3.50^{a} ±0.01	3.47^{a} ±0.03	3.50^{a} ±0.05	3.46^{a} ±0.03	3.50^{a} ±0.05
Left Femur Thickness mm	0.27^{a} ±0.02	0.30^{a} ±0.01	0.27^{a} ±0.03	0.28^{a} ±0.02	0.30^{a} ±0.01	0.30^{a} ±0.01	0.27^{a} ±0.03	0.30^{a} ±0.01

Each value represents the mean ± SE. The mean values with different superscript alphabets indicate significant differences ($P \leq 0.05$) using LSD E2**:** 17α ethinylestradiol –Lig :lignan – FB : flat bread

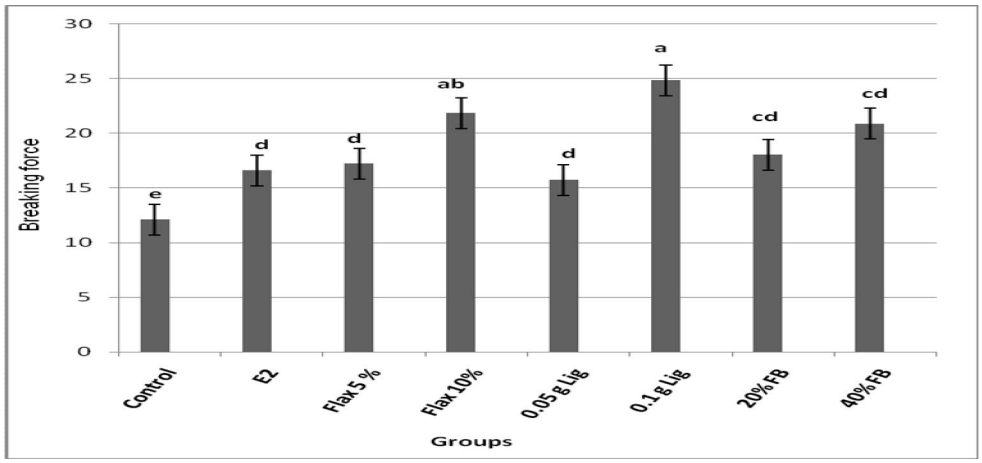

Fig (1) Effect of Flaxseed and its extract supplementation on breaking force

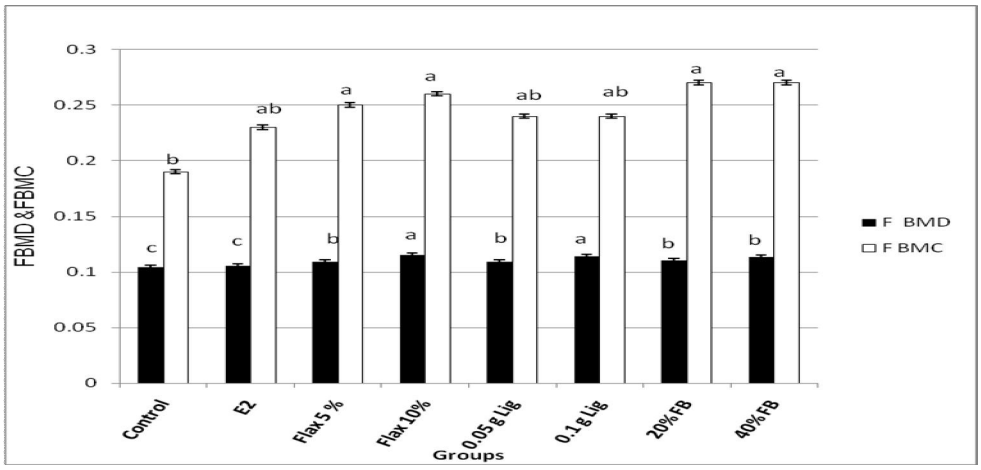

Fig (2) Effect of Flaxseed and its extract supplementation on FBMD &FBMC

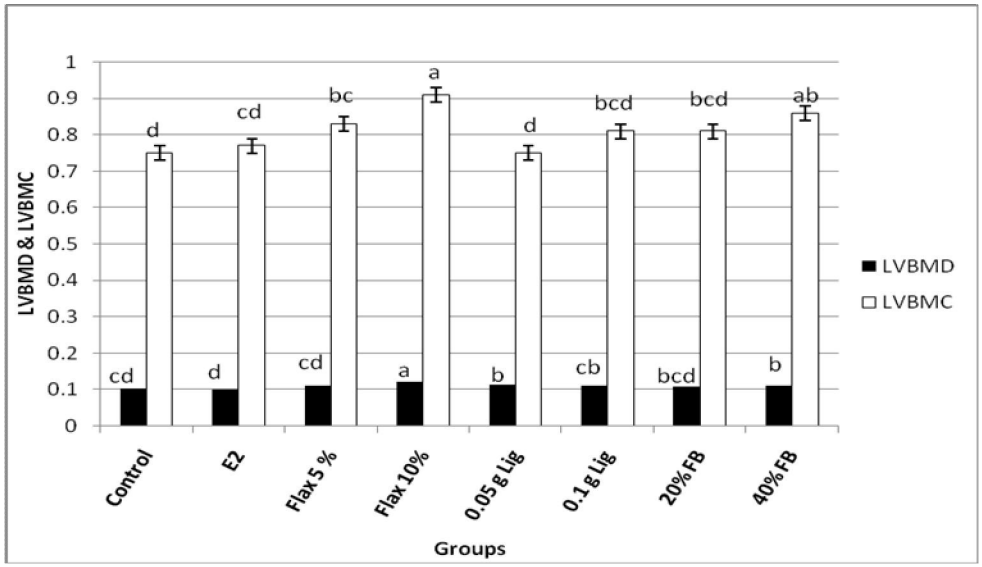

Fig (3) Effect of Flaxseed and its extract supplementation on LVBMD &LVBMC

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