Role of Seeds as a Nutraceutical against Hormone Imbalance and Osteoporosis in Rats.

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ABSTRACT

Hormone deficiency in postmenopausal women is a major risk factor for osteoporosis. The study assessed the effects of Lepidium Sativum (LS) and flaxseed (FS) enriched meals on hormones and osteoporosis in lab ovariectomized rats.

The study included two parts, **the first part**: A total of 40 rats were divided into eight equal groups, each with a weight ranging from 200±10g.Group (1) was a control, while seven groups underwent OVX surgery, while group (2) was fed a basal diet post-ovariectomy, group (3) was fed experimental diets for eight weeks .Rats' body weights were monitored during feeding, urine samples were taken to estimate calcium and phosphorus levels, and blood samples were collected to assess estrogen, progesterone, TSH, calcium, phosphorus, bone Alkaline Phosphatase, and osteocalcin levels. Femur bones were dissected and analyzed for histological and DEXA purposes. The results of Lepidium Sativum and flaxseed have nutraceutical effects that improve body weight gain and uterine weight induced by ovariectomy. These diets increase estrogen and progesterone levels, improved TSH level normalize serum calcium, and decrease urinary excretion of calcium and phosphorus. They also improve bone density in the femur. **The second part:** preparation seven samples of a food product (energy bars) with different percentages of with Lepidium Sativum and Flaxseed Generally in different combinations, the results of total score of sensory evaluation demonstrated that, all the energy bars were acceptable. The study recommends incorporating Lepidium Sativum and flaxseed into diets and food items for hormonal imbalance and postmenopausal osteoporosis treatment in women.

Keywords: Lepidium Sativum; Flaxseed; estrogen; Postmenopausal; osteoporosis.

INTRODUCTION

Hormones are chemical messengers secreted by endocrine glands that control and coordinate various body functions, such as growth, development, metabolism, and reproduction**(Chainy and Sahoo, 2020).**. They come in various forms, including steroids, protein, and amino acid derivatives, which can lead to hormonal diseases **(Santos-Marcoset *et al.*, 2023).** Menopause is a normal physiological event in women, occurring at a median age of 51 years. Perimenopause is irregular menstruation before menopause, with variable duration **(Bhatnagar and Kekatpure, 2022).** However hormone replacement therapy (HRT) is used to relieve menopausal symptoms, with estrogen being taken daily and progestogen added for endometrial protection **(Walker and Shane, 2023).**

Osteoporosis is a bone disorder causing bone loss and increased fracture risk, affecting 10 million Americans and 34 million with low bone density**(Lorentzon *et al.*, 2022).**moreover Women are more susceptible due to thinner bone and faster bone loss during menopause **(Morin *et al.*, 2021).** One in two women and one in four men over 50 will have an osteoporosis-related fracture in their lifetime, with hip and vertebral fractures being the most serious **(Rozenberg *et al.*, 2020).**Osteoporosis causes 1.5 million fractures annually, with 300,000 being vertebral fractures. Treatment options include mesenchymal stem cells, bone-regeneration therapies, and lifestyle changes. Female gender is a strong predictor of osteoporosis, with an estimated 200 million women affected globally, based on age of onset **(Lorentzon *et al.*, 2022) and (Salari *et al.*, 2021)**

Over all, Hormone replacement therapy (HRT) is a synthetic steroid used to treat vasomotor symptoms in women during menopause, affecting 80% of them. It reduces all-cause mortality and risks of coronary disease, osteoporosis, and dementias. However, use declined after the Women's Health Initiative (WHI) trial in 2002 due to side effects **(Langer *et al.*, 2021).**

Lepidium Sativum, a valuable source of nutrition with therapeutic properties, has been investigated for its nutritional profile in recent years **(Painuli *et al.*, 2022).**

Flaxseed, an ancient cultivated crop, is known for its fibers and health benefits **(Grammatikopoulou *et al.*, 2020).** Flaxseed oil, containing 50% to 60% omega-3 fatty acids, including alpha-linolenic acid, has been shown to reduce the risk of various adverse effects **(Casals *et al.*, 2021).** Flax seeds also contain a rich source of ALA and phytoestrogen, which help decrease the risk of cardiovascular diseases (CHD), lower high blood pressure, and other heart-related problems. Additionally, phytoestrogens help normalize hormone levels and reduce the risk of osteoporosis **(Huang *et al.*, 2024).**

Aim of study: Therefore, the present study aimed to investigate Therapeutic Nutritional Effects of Lepidium Sativum and Flaxseed on Estrogen, Progesterone and Osteoporosis in Ovariectomized Experimental Rats.

MATERIALS AND METHODS

MATERIALS:

- The study involved obtaining Lepidium Sativum, flax seeds, starch, corm oil, oat, dates, almond, sesame seeds paste, salt from the local market.
- Casein, vitamins, minerals, cellulose, choline chloride were obtained from Elgomhoria Company, Mansoura, Egypt.
- Forty normal female albino rats from the Nile Center Experimental Research laboratory.
- Kits were provided by Gamma tread Company (GTCO), Cairo, Egypt to measure serum calcium, phosphorous, osteocalcin, TSH, estrogen, progesterone, and urine levels.

METHODS.

Chemical analysis

Calculating the amounts of moisture, protein, fat, and ash using the guidelines provided by the Association of Official Analytical Chemists carried out according to the method of **(A. O. A. C. 2000).**

The variations in carbohydrates (%) = 100 - (water + fat + protein + fiber + ash) was the method used to compute the amount of carbohydrates. Analysis of fatty acids: LS and FS total fatty acid content was determined by adjusting the approved AOAC technique **(A.O.A.C, 1990).** Antioxidant capacity was calculated using the **(McCleary, 2023)** technique.

Experimental design:

Forty normal female albino rats (Sprague Dawley Strain) weighing 200±10g were kept individually in hygienic metabolic cages with a temperature of 25 °C, 50% humidity, and a 12-hour light/12-hour dark cycle. For one week, the rates were fed a basal diet and were given water to help them adjust. Rat experimentation was conducted in compliance with national animal welfare and animal committee regulations. The basal diet was prepared according to the recommended dietary allowances for rats.

(American Institute of Nutrition, AIN) adjusted by **(Reeves *et al.*, 1993).** Basal diet consisted of

14% protein, 10 % sucrose, 5 % corn oil, 0.25% choline chloride, 1% vitamin mixture**(Kraus *et al.*, 1997).**, 3.5 % salt mixture **(Bier *et al.*, 2018).**and 5% fibers(cellules). The Remainder was corn starch up to 100 %.

Table (1): Composition of basal diet (g/kg)

Ingredients	Casein protein	Corn oil	Choline chloride	vitamin Mix.	Salt mix.	cellules	Corn starch	Total amount
G	175	40	2.5	10	35	50	688	1000
%	14%	4%	0.25%	1%	3.5%	5%	66.25%	100

After the period of adaptation on basal diet the rats were divided in two main groups as follow: **The first main group (5rats):** Were given a baseline diet (as a negative control group). **The second main group (35 rats):** were given a baseline diet for three weeks following their ovariectomy treatment in order to promote healing and prevent osteoporosis due to an estrogen deficit. Following that, subgroups of the rats in this group (n=5) were created.

Table (2): The animal's administration for working groups

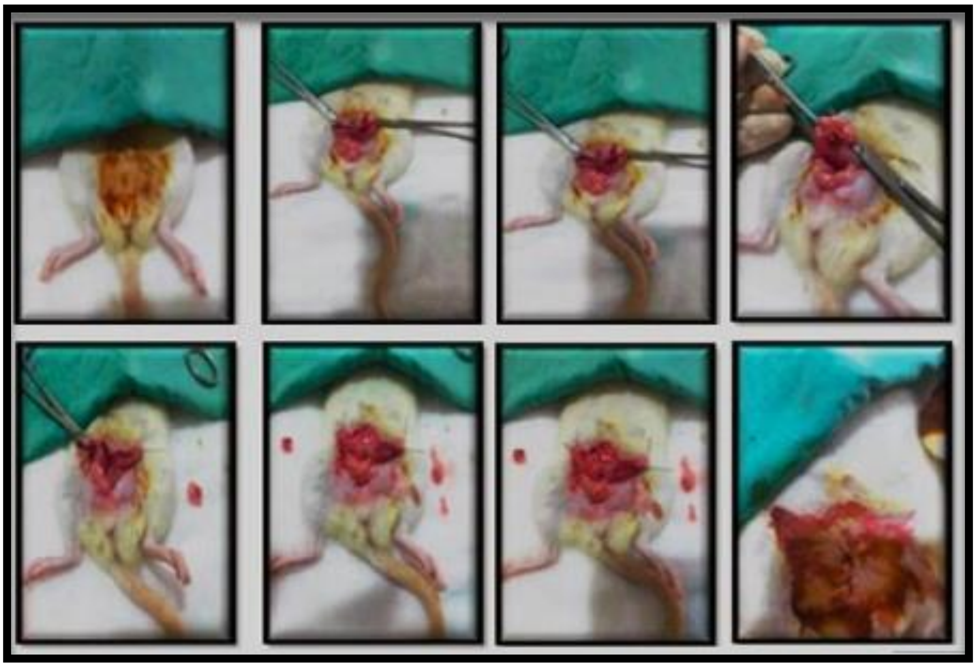
Group NO	Diet and symbol	Description
Group 1	(**NC**) Negative control	Fed on basal diet as Negative control group
Group 2	(**PC**) Positive control	Fed on basal diet as Positive control group
Sub group 1	**G2**+5%LS	Fed on basal diet containing 5%LS
Sub group 2	**G2**+10%LS	Fed on basal diet containing 10%LS
Sub group 3	**G2**+10%FS	Fed on basal diet containing 10%FS
Sub group 4	**G2**+15%FS	Fed on basal diet containing 15%FS
Sub group 5	**G2**+5%LS+10%FS	Fed on basal diet containing 5%LS+10%FS
Sub group 6	**G2**+10%LS+15%FS	Fed on basal diet containing 10%LS+15%FS

(NC) Negative control**, (PC)** Positive control**, (LS)** Lepidium Sativum**, (FS)** Flaxseed

Ovariectomy operation in rats

Anesthesia: Rat ovariectomy under ketamine-xylazine combination anesthesia given via the abdomen (I/P) at a dosage based on the weight of each rat.

Operation: Three centimeter longitudinal incisions were made in the rat's uterus, directly behind the bladder. Each ovary was then located, its ovarian arteries were tied, and the ovary was removed from the abdominal cavity. The muscles and skin were then sutured separately using absorbable sutures in straightforward, continuous patterns. Antibiotic and anti-inflammatory medications were used as part of the post-operative therapy carried out according to the method of (**Khajuria *et al.*, 2012) & (Wronski *et al.*, 1989).**as demonstrated in **Pict (1).**



Pict (1):
Ovariectomy operation in rats.

Biological determination: In order to measure food consumption and body weight growth over the eight-week (8-weeks) experiment, daily food records were made, along with weekly weight records as stated by **(Zhang *et al.*, 2017).**

Body weight gain was determinates using the following equation:

$$\textbf{Body weight gain} = \frac{final\ weight(g) - intitial\ wight\ (g)}{initinal\ wight\ (g)} \times \mathbf{100}$$

Organ: Each rat's uterus was removed, cleansed of sticky material, and weighed using the procedure outlined by **(Walter and Addis, 1939).**

$$\textbf{Organ weight/body weight \%} = \frac{organ\ weight}{final\ weight} \times \mathbf{100}$$

Biochemical analysis:

At the end of experiment, rats were fasted for the entire night, and then scarified by administering an excessive amount of halothane via inhalation anesthetic. Blood was then drawn directly from the heart using a cardiac puncture, with 2 ml going into an anticoagulant tube and the remaining blood going into a blank tube for serum extraction. The blood was centrifuged for 15 minutes at 4000 rpm to extract the serum, which was then frozen at -18°c until biochemical analyses were. Urine samples from the previous day were collected, acidified with 6 Mol HCI, and stored in the refrigerator until biochemical analysis was completed.

Urine samples of the last 24 hour were collected, acidified with 6 Moll HCI and kept in the refrigerator till biochemical analyses. Concentrations of calcium **(Weybrew *et al.*, 1948) and** phosphorus **(Tietz, 1995)** in serum. And urine samples were colorimetric ally determined using specific diagnostic reagent kits and measured on UV spectrophotometer according to **(Parentoni *et al.*, 2001; and Bisergaeva and Sirieva, 2020)**. Serum ALP **(Marsh *et al.*, 1959; and Tietz and Andresen, 1986)** was estimated by colorimetric assay using specific enzyme kits).Serum osteocalcin concentration was measured by enzyme-linked immunosorbent assay according to **Zhang *et al.*, (2021).** Estrogen from blood samples was detected according to **(Gao *et al.*, 2014).** Determination of progesterone was detected according to **(Hoffmann *et al.*, 1973).** Determination of TSH was detected according to **(Thienpont *et al.*, 2013).**

Bone analysis: Following the rats' sacrifice, the right femur was cut free, the soft tissues were taken out and weighed, and the length was measured with a Vernier caliper. Additionally, volume was computed using the Archimedes method **(Hughes, 2005).** Additionally, samples were preserved in aluminum foil until the DEXA scan was performed **(Zou *et al.*, 1997).**

Histopathological examination: When the experiment came to the end, the animals were sacrificed, and the femur shafts were taken out, dipped in glutaraldehyde, and then, after four hours, left to decalcify in EDTA solution for twenty days. Using a standard procedure, paraffin (5 µm) tissue slices were cut from the middle shaft of the femur. Sections were viewed under a light microscope after being stained with hematoxylin and eosin **Modi *et al.*, (2016) and Palmer *et al.*, (1999).**

Sensory evaluation:

Energy Bars (7samples) supplemented with Lepidium Sativum and flaxseed combination evaluated for color, odor, texture, taste and overall acceptability by twenty five person .The evaluation was carried out according to the method of **(A.A.C.C. 2002).**

Processing of Energy bars:

Ingredients

Control Energy bars was made from 1½ cup rolled oats,½ cup Tahini, ⅓ cup dates, ⅓ cup almond ,1teaspoon vanilla extract,¼ teaspoon salt, Seeds with different percentages (Lepidium Sativum- crushed flax seeds).

Directions

Stir oats and all ingredients together in a bowl. Roll into bars and refrigerate until firm, at least 30 minutes.

Preparation of Energy bars:

Different sample of Energy bars were classified as follows:

1. **Control:**
2. **Different Formulas:**

a- **Treatment:** 5% Lepidium Sativum.
b- **Treatment:** 10% Lepidium Sativum.
c- **Treatment:** 10% flaxseed.
d- **Treatment:** 15% flaxseed.
e- **Treatment:** 5% Lepidium Sativum + 10% flaxseed.
f- **Treatment:** 10% Lepidium Sativum + 15% flaxseed.

Statistical analysis: A computer was used to perform statistical analysis on the data. The findings were shown as mean ± standard deviation ("S.D") and were compared between two sets of numerical (parametric) data using a one-way analysis of variance (ANOVA) test. Next, post-hoc Tukey was run. According to, a P value of 0.05 was deemed statistically significant**(Armitage and Berry, 2008).**

RESULT AND DISCUSSION

Chemical composition values of Lepidium Sativum and Flaxseed:

Data in Table (3) show that energy, total lipid, ash, carbohydrate, calcium, magnesium, more obviously increased in Lepidium Sativum compared to flaxseed as expected, flaxseed had high content of protein, phosphorous and Isoflavones in comparing with Lepidium Sativum.

Lepidium Sativum and flaxseed are good sources for the different food components including (protein; Total lipid (fat); Ash; Carbohydrate; Ca; Fe; P; Such data were agree with that obtained by **Mohamed *et al.*, (2023),Tufail *et al.*, (2024)** and **Abdulrahman *et al.*, (2024)** which they reported that, Lepidium Sativum (LS) and flaxseed (FS) are a rich sources of protein, fatty acids, Ca, P and active nutrients such as antioxidants and Isoflavones **Zhang *et al.*, (2023)** and **Gandova *et al.*, (2023)**

Table (3): Chemical composition values of Lepidium Sativum and Flaxseed:

Components	Lepidium Sativum(LS)	Flaxseed(FS)
Chemical composition		
Water (g)	3.46	6.96
protein (g)	22.4	18.3
Total lipid (fat) (g)	22.0	42.2
Ash (g)	5.5	3.72
Carbohydrate (g)	32.85	28.9
Total saturated fatty acids (g)	0.023	3.66
Minerals content		
Calcium, Ca (mg)	243.7	255
Iron, Fe (mg)	1.30	5.73
Magnesium, Mg (mg)	38.00	392
Phosphorus, P (mg)	590.7	642

(LS) Lepidium Sativum**, (FS)** Flaxseed

Biological determination:

Effects of Lepidium Sativum and Flaxseed on Feed intake (g/day/rat) and BWG% and food efficiency Ratio FER:

The effect of Lepidium Sativum and Flaxseed with different percentage on the mean value of the feed intake (g/day/each rat) and on body weight gain% of ovariectomized rats suffering from hormone imbalance and osteoporosis presented in table (4)

Feed intake:

Data in table (4) and showed that, the mean value of feed intake of healthy rat Control Negative about 18.8g/day/rat, while the mean value of feed intake in ovariectomized rats suffering from Osteoporosis decreased to 13.1g/day each rat in Positive control. Treated rat with Lepidium Sativum and Flaxseed increased the mean value of feed intake about 18.1g,16.8g,17.6g,17.1g,16.4g and17.9g for 5%LS, 10%LS, 10%FS, 15%FS, 5%LS+10%FS and 10%LS+15%FS respectively treated groups if compare that with Positive control.

Treating ovariectomized rats suffering from hormone imbalance and osteoporosis rats with Lepidium Sativum and Flaxseed with different percentage led to slight increase in the mean value of feed intake, as compared to the Control Positive group. While the mean values of feed intake in rat consumed 5%LS, 10%LS, 10%FS, 15%FS, 5%LS+10%FS and 10%LS+15%FS respectively going to be nearly to the control negative intake. The highest increase in the mean value of the feed intake recorded for the group feeded on diet containing the combination (5%LS) followed by the group fed on diet containing (10%LS-15%FS).

Body weight gain%:

The data in this table (4) revealed that, body weight gain% of the control positive group which ovariectomized and suffering from hormone imbalance and osteoporosis decreased significantly $p<0.05$, as compared 12.80±3.01the control positive to (5.05±2.01, 7.80±2.08, 4.50±2.00, 6.03±2.05, 5.00±2.01 and 5.60±2.02), (5%LS, 10%LS, 10%FS, 15%FS, 5%LS+10%FS and 10%LS+15%FS) respectively, feeding rats with Lepidium Sativum and flaxseed with different percentage decreased the mean value of body weight gain% to 7.85%±1.12 and 6.73%±1.64 than that of the control negative group.

Treated ovariectomized rats suffering from osteoporosis rats with Lepidium Sativum and flaxseed with different percentage led to significant changes in BWG%. On the other hand, BWG% of the groups which treated with different levels of FS showed significant increase as compared to the control positive group.

Results in this table (4) Showed significant changes in body weight gain% between the groups which treated with (Lepidium Sativum and flaxseed. On the other hand, the highest decrease in body weight gain % of all experimental groups recorded for the group fed on diet containing (10%FS) As compared to other experimental groups. Treated rats with 5%LS, and the combination (10%LS, 10%FS, 15%FS, 5%LS+10%FS and 10%LS+15%FS) revealed a significant decrease ($P\leq0.05$) of food efficiency Ratio FER by comparing with the positive control group.

Uterus weight/ body weight %:

The results in table (4) and the fig (2) showed that, all treated ovariectomized rats suffering from osteoporosis with diet fortified with Lepidium Sativum and Flaxseed showed a significant decrease ($p<0.05$) in uterus weight /body weight%, as compared to the control negative group.

These findings were consistent with those reported by **Rashnou *et al.*, (2023); Mohamed *et al.*, (2023); and Sabet *et al.*, (2024).** Flaxseed consumption has been linked to weight loss and improved blood lipids in people, as well as anti-oxidative capabilities in animals. These studies concluded that flaxseed consumption had an influence on blood sex hormones, lipids, and LDL oxidation in postmenopausal women.

On the other hand, **(Embafrash Berhe *et al.*, 2023)** demonstrated that, despite a large increase in food consumption compared to OVX rats, the body weight of Lepidium Sativum fed rats decreased dramatically. This suggests that by increasing food consumption, Lepidium Sativum can manage body weight growth. On the other hand, the body weight of the OVX / Lepidium Sativum fed group was the same as that of the daily diet fed group (CN-).

According to **Whittle *et al.*, (2024),** estrogen insufficiency is a major cause of postmenopausal bone loss and, together with obesity, contributes to age-related bone loss. When estrogen levels were reduced, OVX gained weight and osteoclasts formed, but other groups treated with seeds improved. In contrast, uterine weight decreased in the OVX group. And had a somewhat significant rise on other treated groups, according to these results, which were almost identical to those reported by **Nowak and Jeziorek (2023).**

On the other hand, **Yang *et al.*, (2021) and Yang *et al.*, (2023)** Estrogen is mostly generated in the ovaries. Anything that affects the ovaries will eventually impair estrogen production. Obesity rates rise after menopause, increasing the risk of osteoporosis, metabolic and cardiovascular illnesses. Estrogen has been linked to increased anti-obesity and anorectic effects. Hormone replacement therapy (HRT) is commonly administered to menopausal women to alleviate postmenopausal symptoms, and it has been demonstrated to be useful in the prevention of obesity and metabolic syndrome in postmenopausal women. A flaxseed-rich diet, as an alternative to HRT, has been found to improve the health of menopausal women because flaxseed isoflavones apparently serve as ER modulators. Therefore, estrogen level improves hormones level and uterus weight.

Alshafei *et al.*, (2020) found that a fortified diet with Lepidium Sativum and flaxseed improved food intake and body weight increase due to their anti-obesity and blood lipid-normalizing effects.

According to the findings of **Ibrahim *et al.*, (2020),** meals enriched with Ca and vitamin D prevented ovariectomy-induced increases in body weight gain and decreases in uterine weight, restoring (CN-) rats' body and uterine weights to near-normal levels. Furthermore, estrogen was found to enhance the vascularity, growth, and weight of the uterus in rats and mice. The reduction in uterine weight caused by ovariectomy may be related to estrogen insufficiency in (OVX) rats.

The same finding was previously reported by ***Zhao et al.*, (2023),** who discovered that bilateral ovariectomy in rats dramatically boosted body weight gain while decreasing uterine weight.

Toulabi *et al.*, (2022) found a significant increase in relative uterine weight in phytoestrogen-fed groups, confirming our results and reducing lipid tissue around the uterus.

Table (4): Effect of Lepidium Sativum and Flaxseed on Food intake (g/day/rat) and BWG% and food efficiency Ratio FER of rats suffering from hormone imbalance and osteoporosis

Parameters / Groups	Feed intake (g/day/rat)	BWG%	FER	Uterus Weight (g)	OWG%
(NC-)Negative control	18.8 ±1.56[a]	3.60±1.95[cd]	1.38 ± 0.15[a]	0.55±0.082[a]	0.29%
(PC+) Positive control	13.1 ±0.85[c]	12.80±3.01[a]	0.75 ±0.07[bc]	0.13±0.039[c]	0.06%
5%LS	17.1 ±1.15[b]	5.05±2.01[bc]	0.93 ± 0.16[b]	0.15±0.061[b]	0.07%
10%LS	16.8 ±0.53[bc]	7.80±2.08[b]	0.93 ± 0.09[b]	0.17±0.065[b]	0.08%
10%FS	17.6 ±1.[21ab]	4.50±2.00[c]	0.82 +0.17[bc]	0.15±0.063[b]	0.08%
15%FS	17.2±1.16[b]	6.03±2.05[b]	0.66± 0.16[cd]	0.15±0.062[b]	0.08%
5%LS+10%FS	16.4±0.52[bc]	5.00±2.01[bc]	0.53± 0.13[de]	0.16±0.066[b]	0.08%
10%LS+15%FS	17.9 ±1.[21ab]	5.60±2.02[bc]	0.41 ± 0.13[e]	0.17±0.069[b]	0.08%

(NC) Negative control, (PC) Positive control, (LS) Lepidium Sativum, (FS) flaxseed
Values are expressed as means ±SD.
The mean value was significant at ($P<0.05$)

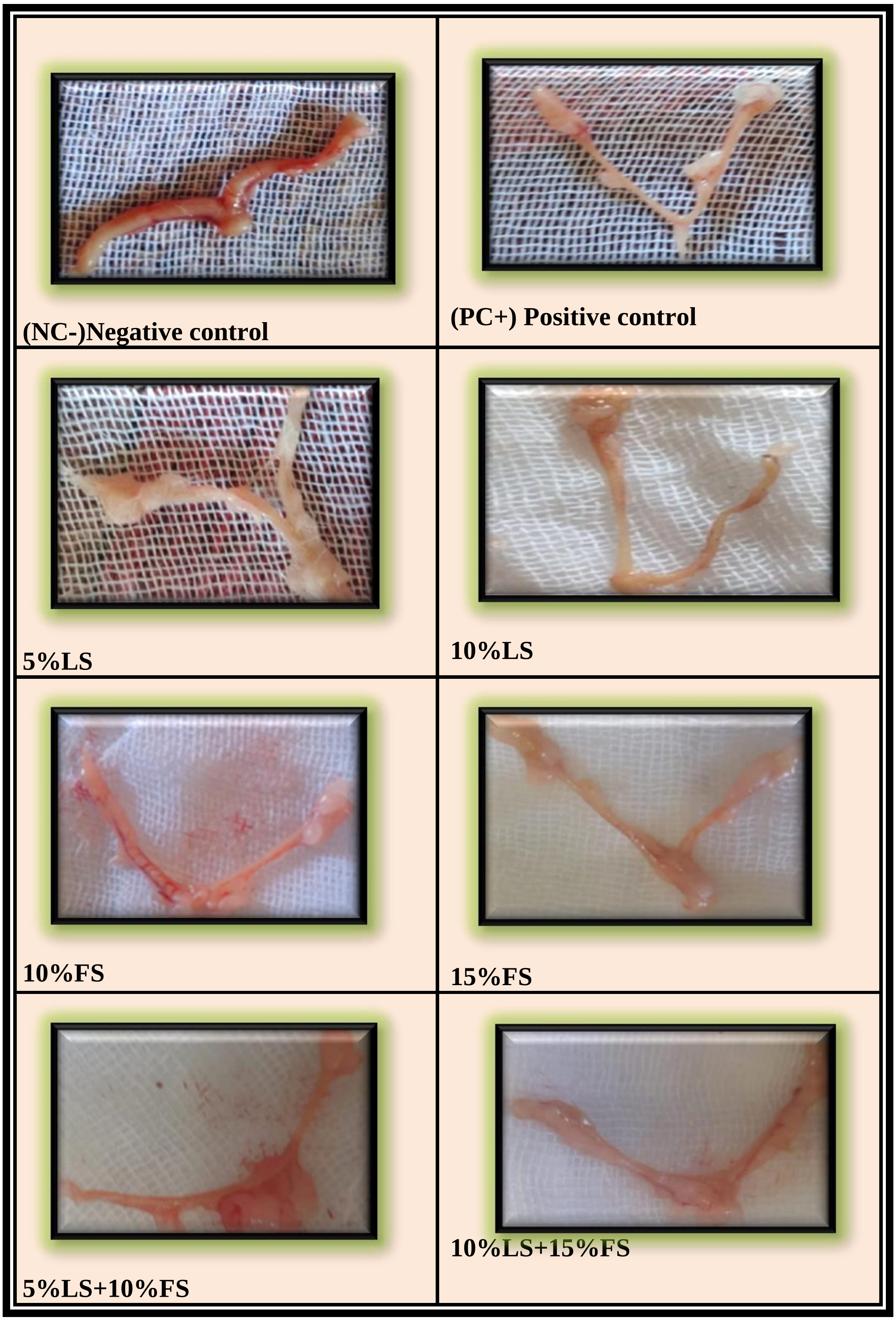


Fig (2): uteruses in rats at the end of experiment.

Biochemical analysis:

Data on Table (5) show the Effect of Diets enrich with LS and FS on blood test (serum calcium (mg/dl); serum phosphorus (mg/dl); ALP (u/l) and OC (mg/dl) of rats suffering from hormone imbalance and osteoporosis.

Blood test:

Serum calcium and phosphorus

Calcium, a key component of the bone skeleton, plays a crucial function in osteoporosis prevention as it correlates with bone mineral density (BMD) and overall bone health **(Abdalbary *et al.*, 2022).** According to **Victor, (2023),** the calcium-to-phosphorus ratio has a crucial role in predicting bone quality and osteoporosis.

Results of feeding ovariectomized rats on diets containing different levels of Lepidium Sativum seeds and Flaxseed on serum calcium and phosphorus were illustrated in the table (5). It could be noticed that untreated osteoporotic group (PC+) recorded significant decreases in serum calcium and phosphorus (mmol/l) as compared them with the healthy control group (NC-) (12.36 ± 0.21and 9.46 ± 0.32vs. 7.36 ± 1.36and 5.83 ± 1.25 mmol/l, respectively), Treating ovariectomized rats with supplemented diet 5% LS, %10 LS, 10% FS, 15 % FS, 5% LS. + 10% FS and 10% LS. + 15% FS improved the levels of serum calcium and phosphorus in rats suffering from hormone imbalance and osteoporosis. The highest increase in serum calcium and phosphorus levels appeared in the osteoporotic group fed on diet supplemented with 5% LS. + 10% FS recorded non-significant changes as compared to the control negative group.

These findings are consistent with those reported by **Bukhari *et al.*, (2018),** who found that L. Sativum improves bone structure and reduces symptoms associated with arthritis and body system inflammation, with significant changes in BMD and improved T-Score at baseline after treatment with L. Sativum. **Massey and Whiting, (2020)** reported that **Flaxseed** increased blood calcium levels. **Tinker *et al.*, (2021)** found that **Lepidium Sativum** seed oil boosted mineral and vitamin D concentrations while also improving histological structure and bone thickness in osteoporotic rats. **Nicoli *et al.*, (2021) and Park *et al.*, (2021)** found that L. Sativum seeds increase bone healing and might be employed as a supplementary or alternative therapeutic osteogenic agent in bone fracture treatment.

Alkaline phosphatase (ALP):

Results in this table(5) revealed that, treating OVX rats suffering from **hormone imbalance and osteoporosis** with diet enrich with LS and FS showed a significant decrease ($P < 0.05$) ALP, as compared to the CP group which fed on basal diet only. As a result, the mean values of mean ±SD of ALP in the CP group increased ($P < 0.05$) compared to CN values.

In this regard, **(Baskar and Dharman, 2021)** found that ovariectomy substantially elevated serum total. ALP activity increased considerably in OVX animals compared to the control Negative group. Both treated groups with flaxseed and SS at 10% showed a substantial reduction in ALP activity ($p < 0.05$).

In other words **(Hutomo et al., 2021).** According to the study, phytoestrogens have the greatest potential for preventing bone loss by lowering BMD loss while simultaneously inhibiting ALP and OC **Rai et al., (2021) and Tariq et al., (2021).**

Osteocalcin (OC):

Results in this table (5) revealed that, treating ovariectomized rats suffering from osteoporosis with diet fortified with Lepidium Sativum and Flaxseed showed a significant decrease ($p<0.05$) (OC), as compared to the control positive group which fed on basal diet only.

As a result, it could be observed that, the mean values mean ±SD of (OC) in the control positive group increased ($p<0.05$) as compared with control negative which are 1198.0 ± 161.0for (PC+) vs 793.66 ± 48.21 (NC-).

Data revealed that, statistically significant differences between the mean values of osteocalcin where ($P<0.05$). In OC which observed between the groups fed on diet fortified with Lepidium Sativum and Flaxseed. The best results in OC were recorded for the groups treated with (10%LS+15%FS), As. these treatments showed significant decrease, as compared to the other treated groups.

According to **Moon *et al.,* (2021)**, Lepidium Sativum down-regulates a few genes linked to osteoclastgenesis. Lepidium Sativum therapy was used to study the expression of osteoclastgenic genes, and the results showed that Lepidium Sativum inhibits the development of human osteoclasts. Effectively suppressing the human osteoclast development process, lignan lowers resorption activity as a result. This discovery, which demonstrates how Lepidium Sativum promotes osteoblast differentiation in both human osteoblast cell lines and mesenchymal stem cells, highlights Lepidium Sativum as a potentially valuable phytochemical agent for bone cell metabolism, as evidenced by the drop in serum OC levels in Lepidium Sativum-treated groups.

However, regardless of ovarian hormone status, flaxseed was shown to increase cortical bone biomechanical characteristics in female low-fit rats **Ugurlu *et al.,* (2022) and Naguib *et al.,* (2022).** Flaxseed dramatically increased stiffness and whole-bone strength. Moreover, flax seed tended to boost tissue-level strength and raised tissue-level stiffness. Additionally, the OC levels in the treated rats significantly decreased, which is consistent with this study findings.

Table (5): Effect of Lepidium Sativum and Flaxseed on serum calcium and serum phosphorus of rats suffering from hormone imbalance and osteoporosis:

Parameters / Groups	serum calcium (mg/dl)	serum phosphorus (mg/dl)	b-ALP(U/L)	Osteocalcin(mg/l)
(NC-)Negative control	9.46 ± 0.32[b]	7.36 ± 1.36[a]	487.39 ±32.05[c]	793.66 ± 48.21[b]
(PC+) Positive control	12.36 ± 0.21[a]	5.83 ± 1.25[c]	786.02±37.97[a]	1198.0 ±161.0[a]
5%LS	8.93 ± 0.15[c]	6.06 ±0.80[bc]	215.96±35.45[e]	882.66 ± 69.15[b]
10%LS	9.2 ± 0.35[ab]	6.53 ± 0.83[b]	411.33 ± 11.21[cd]	584.66 ± 29.02[c]
10%FS	9.03 ± 0.015[bc]	6.16 ± 1.2[bc]	663.26±38.79[ab]	579.0 ± 83.14[c]
15%FS	8.9 ± 0.4[c]	7.06 ±0.37[ab]	562.33 ± 20.6[b]	468.0 ± 68.94[d]
5%LS+10%FS	9.13 ± 0.32[ab]	7.03 ±1.36[ab]	597.21 ± 12.2[b]	909.33 ± 140.15[ab]
10%LS+15%FS	9.07 ± 0.42[bc]	6.83 ± 0.55[b]	390.03±14.25[d]	707.0 ± 45.17[bc]

(NC) Negative control**, (PC)** Positive control**, (LS)** Lepidium Sativum, **(FS)** flaxseed
Values are expressed as means ±SD.
The mean value was significant at **(P<0.05)**

Hormone blood test (estrogen-progesterone-TSH):

Estrogen:

Results in this table (6) showed that, treating ovariectomized rats suffering from hormone imbalance and osteoporosis with diet enriched with Lepidium Sativum and flaxseed showed a significant increase ($p<0.05$) in estrogen, as compared to the control positive group which fed on basal diet only.

As a result, it could be observed that, the mean values mean ±SD of estrogen in the control positive group decreased ($p<0.05$) as compared with Control negative which are 50.12 ± 19.62 (CP+) vs 154.99 ± 6.35for (CN-).
Data stated that there were statistically significant variations between mean estrogen values where ($p<0.05$). Estrogen was observed between diet-fed groups fortified with Lepidium Sativum and flaxseed. The best results of estrogen level were reported for treatment groups (15 % FS), as a related. compared with other treated groups, this therapy showed a substantial improvement.

According to **Tanideh *et al.*, (2021),** women participating in this study took in 50 grams of flaxseed powder every day for five weeks. This improved blood cholesterol levels in addition to increasing estrogen action. An excellent source of phytoestrogens is flaxseed. It is confirmed that consuming flaxseed on a regular basis increases estrogen activity in postmenopausal women **(Vlčková *et al.*, 2022).**

According to **(Pinkerton, 2020),** estrogen is the most powerful inhibitor of osteoclastic bone resorption, and as such, estrogen insufficiency is a significant risk factor in the etiology of osteoporosis. Because of an estrogen shortage that increases the production of osteoclasts and increases bone loss from osteolysis, the bilateral ovariectomy in rats resulted in considerable reductions in uterine weight, bone mineral content, density, and biomechanical strength. According to **Genazzani *et al.*, (2021) and Matar *et al.*, (2021)** ERT is frequently used to treat postmenopausal osteoporosis. Furthermore, a deficiency in critical micronutrients for bone health, calcium and vitamin D, raises the risk of osteoporosis by increasing the incidence of bone loss **Vigneswaran and Hamoda, (2022)**.

According to research by **Whitten *et al.,* (2020) and Błaszczuk *et al.,* (2022)** micronutrients like isoflavones should be acknowledged as a novel class of substances that support calcium homeostasis in premenopausal women. Aside from that, isoflavones function similarly in postmenopausal women who are estrogen deficient.

The observed results according to **Lecováe *et al.,* (2022)** indicate that flaxseed as a 10% diet supplement fed for six weeks applied its supporting effects on the endometrium thickness (including luminal epithelium, lamina propria and number of endometrial glands) as the effect on the steroid hormone receptors expression. Moreover, its effect on the myometrium was supported by binding phytoestrogens to nuclear ERβ receptors which is balanced ovarian hormones.

<u>progesterone :</u>

Results in this table (6) showed that, treating ovariectomized rats suffering from hormone imbalance and osteoporosis with diet enriched with Lepidium Sativum and flaxseed showed a significant increase ($p<0.05$) progesterone, as compared to the control positive group which fed on basal diet only.

As a result, it could be observed that, the mean values mean ±SD of progesterone in the Control Positive group decreased ($p<0.05$) as compared with Control negative which are 1.32 ± 0.13 (PC+) vs 3.27 ± 0.33for (NC-).

Data in Table (6) stated that there were statistically significant variations between mean progesterone values where ($p<0.05$). Progesterone was observed between diet-fed groups enriched with Lepidium Sativum and flax seed. The best results of progesterone level were reported for treatment groups (15 % FS.) and (10% LS + 15% FS) respectively as a related. Compared with other treated groups, this therapy showed a substantial improvement.

According to research by **Seifert-Klauss, (2020); Santoro *et al.,* (2021) and Vlčková *et al.,* (2022)** the interaction between ovulation, progesterone and bone metabolism is complex. Accumulating physiological and clinical evidence however point towards a role for ovulation and progesterone in enhancing bone formation and limiting bone resorption.

In this regard, **El-Tarabany *et al.,* (2020) and Vlčková *et al.,* (2022)** found that it could be concluded that supplementing diets by folic acid or flaxseed improved significantly the blood biochemical indices and the progesterone profile during Postmenopause stages. Interestingly, the Fs supplement increased significantly the female hormone rate balance as compared with the CN group.

Obeid *et al*., (2020) and Shah *et al*., (2021) declared that Lepidium Sativum seeds have a great importance for increasing the evolution and expansion of the mammary glands and increasing the level of sex hormones in female rats during three physiological stages: virgins, pregnancy and lactating and Postmenopause seeds noted that Lepidium Sativum seeds contain many alkaloids and flavonoids Some of these alkaloids may have action on different endocrine glands and influence secretion of hormones by those glands. Presence of these alkaloids also play significant role in hormonal improve for the treated experimental animals.

TSH :

Results in this table (6) showed that, treating ovariectomized rats suffering from hormone imbalance and osteoporosis with diet enriched with Lepidium Sativum and flaxseed showed a significant increase ($p<0.05$) TSH, as compared to the control positive group which fed on basal diet only. As a result, it could be observed that, the mean values mean ±SD of TSH in the Control Positive group decreased ($p<0.05$) as compared with Control negative which are 4.12 ± 0.46 (PC+) vs 1.03 ± 0.12 for (NC-).

TSH was observed between diet-fed groups enriched with Lepidium Sativum and flax seed. The best results of TSH level were reported for treatment groups (10% LS+ 15% FS) and (5% LS+ 10% FS) respectively as a related. Compared with other treated groups, this therapy showed a substantial improvement.

From the data conformed in **Avcı and Erdoğan, (2017); Sciarrillo *et al*., (2018)and El-Emary, (2021)** studies Groups treated with extract seeds of L. Sativum showed significant decline T3 and T4 levels together with significant increase in TSH concentrations as compared to control group. That administration of L. sativum seed extract decreases the levels of the thyroid hormones indicating its thyroid inhibitory nature similar to other plants.

Matar *et al*., (2021) showed that almost of treated groups induced increase within normal range (TSH) hormone, (T3) and (T4) at post treatment compared to pretreatment between groups especially control group and the differences between groups were significant .The greatest increase of TSH, T3 and T4 hormones were obtained by supplementation with flaxseed powder then followed by women who have been low carbohydrate low protein diet compared to the control group, this was agreed with **(Heidari *et al*., 2020) .**

The greatest decrease of TSH, T3 and T4 was obtained by control group .The increase induced in TSH, T3 and T4 hormones in all treated groups may be attributed to their antioxidant properties of their bioactive constituents. Since, adding flaxseed, intermitting fasting and low carbohydrate low protein diet caused a significant decrease of testosterone, increased estradiol concentration, also each of growth hormone and TSH hormones increased significantly **(Kazemizadeh *et al*., 2023).**

Table (6): Effect of Lepidium Sativum and Flaxseed on hormone blood test (estrogen-progesterone-TSH) of rats suffering from hormone imbalance and osteoporosis:

Parameters / Groups	Estrogen (Pg/ml)	progesterone(ng/ml)	TSH(ulU/ml)
(NC-)Negative control	154.99 ± 6.35^{a}	3.27 ± 0.33^{a}	1.03 ± 0.12^{c}
(PC+) Positive control	50.12 ± 19.62^{d}	1.32 ± 0.13^{c}	4.12 ± 0.46^{a}
5%LS	95.53 ± 13.54^{c}	1.98 ± 0.12^{b}	2.11 ± 0.21^{b}
10%LS	120.03 ± 19.48^{b}	2.47 ± 0.12^{ab}	2.52 ± 0.26^{b}
10%FS	79.89 ± 21.29^{a}	1.52 ± 0.22^{bc}	3.56 ± 0.61^{ab}
15%FS	1٥8.95 ± 24.09^{a}	3.٥1 ± 0.44^{a}	0.53 ± 0.13^{d}
5%LS+10%FS	97.12 ± 17.29^{bc}	1.79 ± 0.11^{b}	1.48 ± 0.11^{bc}
10%LS+15%FS	135.4 ± 8.58^{ab}	2.66 ± 0.19^{ab}	1.23 ± 0.11^{bc}

(NC) Negative control**, (PC)** Positive control**, (LS)** Lepidium Sativum, **(FS)** flaxseed
Values are expressed as mean ±SD.
The mean value was significant at **(P<0.05)**

Urine tests:

Urine calcium:

Table (7) illustrated that, the mean values of Urine calcium in the Control Positive group increased (P<0.05) as compared with Control Negative which are 11.67 ± 0.71for (PC+) vs 8.96 ± 0.53for (NC-).

in other wise Data presented showed that all treated ovariectomized rats suffering from Osteoporosis with diet Fortified with Lepidium Sativum and Flaxseed showed a significant decrease (p<0.05) in urine calcium, as compared to the Control Positive group.

On other hand, treating ovariectomized rats suffering from Osteoporosis with (10%FS) and combination of (10%LS+15%FS) recorded the best result in urine calcium (mg/dl) revealed a significant decrease (p<0.05) when compared to other groups.

Urine phosphorus

Illustrated outcomes in table (7) show, the Therapeutic Nutritional Effects of Lepidium Sativum and Flaxseed on urine phosphorus of rats suffering from hormone imbalance and osteoporosis. Rats which were fed on fortified diet led to significant decrease (P<0.05) in urine phosphorus as compared to the positive control.

As a result, on table (7) it could be observed that, the mean values mean ±SD of Urine phosphorus in the Control Positive group increased (p<0.05) as compared with Control Negative which are 7.05 ± 0.38for (CP+) vs 4.66 ± 0.29 for (CN-).

Data in table (7) revealed that, statistically significant differences between the mean values of Urine phosphorus where (p<0.05). Urine phosphorus was observed between the groups fed on diet enriched with Lepidium Sativum and Flaxseed. The best results in serum phosphorus was recorded for the groups treated wit (10%LS) and combination of (10%LS+15%FS) respectively, As. this treatments showed significant decrease, as compared to the other treated groups.

According to the current investigation **(Sakran *et al.,* 2024),** the bone resorption marker urine test was dramatically reduced by LS isoflavone supplementation. In contrast to other bone turnover indicators, LS isoflavones had a mild and non-significantly significant effect on the urine test.

According to **Hafiz *et al.,* (2022)** bone resorption and formation peaked 5–10 years after menopause, and among bone resorption indicators, serum is more helpful than urine. It is crucial to conduct serial measurements of bone turnover markers (BTM) and BMD within a decade following menopause in order to accurately diagnose and treat postmenopausal osteoporosis. Bone mineral density (BMD) and serum osteocalcin (S-OC) are the best markers of bone development. The goal of **Watanabe *et al.,* (2020**) investigation was to determine whether an osteoporosis diagnosis could be made using a urine test. The outcome indicates that, because urine is a bone resorption biomarker with some pre-analytical and biochemical variability that might affect the results, it can very well be called a diagnostic test but not a gold standard diagnostic test. In addition to the highest quality Dexa, it can be used as a screening test and adjuvant for osteoporosis diagnosis.

As a Conclusion results were in accordance with that of **(El-Salam *et al.,* 2019); (Rafińska et *al.,* 2019) and (Ahmad *et al.,* 2021)** who found that Lepidium Sativum are a very good source of magnesium and Calcium. Beside its content of lignan which has avital role in bone recovery as a Phyto estrogen.

Moreover the results of present work were in agreement with **Mattioli *et al.,* (2017); Peirotén *et al.,* (2020); Landete, (2022) and Bonilla *et al.,* (2024)** in that flaxseed and is a dietary source of isoflavones that is nutritionally significant. Flaxseed isoflavones have mild estrogenic activity, bind to estrogen receptors, and share structural similarities with estrogen. The potential of flaxseed components to prevent chronic diseases, including cancer, heart disease, and bone health, has drawn attention. These are soy isoflavones' estrogenic qualities and how well they work to stop bone loss in both premenopausal and postmenopausal women. Furthermore, flaxseed reduces calcium excretion when it replaces animal protein, according to a number of human researches.

The outcomes were almost identical to those reported by **Abdallah *et al.,* (2020), El-Haroun *et al.,* (2020), and Elshal *et al.,* (2023).** Lepidium Sativum stops bone loss caused by osteoporosis regardless of weight or length of therapy. In persons of normal weight, they raise BMD; in those who are overweight or obese, they reduce bone resorption. Longer than a year is probably required impacting BMD, even if short-term isoflavone ingestion may slow bone resorption. Isoflavones also seem to improve bone, regardless of the subject's ethnicity or dosage.

Table (7): Effect of Lepidium Sativum and Flaxseed on Urine Calcium and Urine phosphorus of rats suffering from hormone imbalance and osteoporosis:

Parameters / Groups	Urine calcium (mg/dl)	Urine phosphorus (mg/dl)
(NC-)Negative control	8.96 ± 0.53[b]	4.66 ± 0.29[b]
(PC+) Positive control	11.67 ± 0.71[a]	7.05 ± 0.38[a]
5%LS	10.38 ± 0.23[ab]	5.76 ± 0.18[ab]
10%LS	6.23 ± 0.33[c]	4.01 ± 0.23[bc]
10%FS	8.42 ± 0.16[b]	5.99 ± 0.26[ab]
15%FS	7.42 ± 0.43[bc]	5.16 ± 0.32[b]
5%LS+10%FS	7.94 ± 0.17[bc]	5.8 ± 0.25[ab]
10%LS+15%FS	9.45 ± 0.4[ab]	3.93 ± 0.55[bc]

(NC) Negative control**, (PC)** Positive control**, (LS)** Lepidium Sativum, **(FS)** flaxseed
Values are expressed as means ±SD.
The mean value was significant at **(P<0.05)**

Histopathological examination of femur bone:

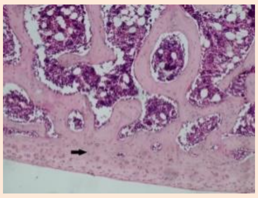
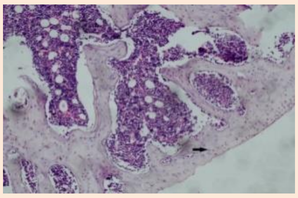
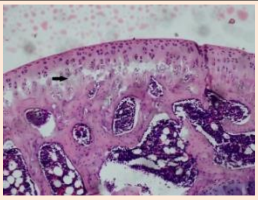
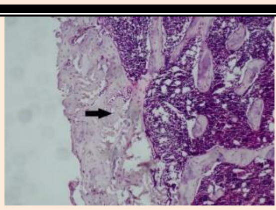
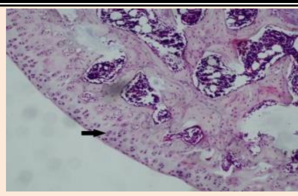
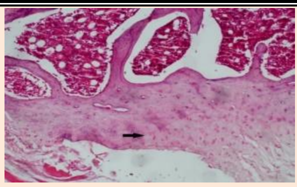
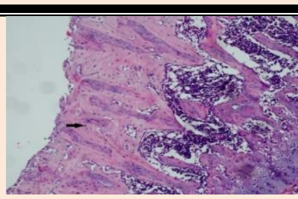
The histological results of this study demonstrated that ovariectomy decreased the thickness of the cortical compact bone in the middle shaft of the femur and the trabeculae in cancellous bone in the head of the femur bone, as shown in (Pict 3). Histochemical analysis revealed new bone growth in sections of rats treated with LS+FS. The greatest findings were discovered in sections of rats treated with a combination of the (10%LS+15%FS) as indicated in (Pict 3), which had a greater ameliorating impact on ovariectomy-induced osteoporosis than other groups as shown in (Pict 3).

Histopathological and morphometric findings Histological sections of the middle shaft of femur from rats of the control negative CN group showed that bone tissue was of the compact type, covered by two layers, the periosteum located externally, which is a dense connective tissue, and the endosteum, a thin cell-rich connective tissue, lining the internal surface of the bone facing the bone marrow cavity Within the bone matrix show in (Pict 3).

As noted in (Pict 3) the compact bone tissue, revealed a considerable reduction of the number of osteoblasts and osteocytes and, specifically, at osteons internal blades (CP+).

Furthermore, most osteocytes seemed weak or missing, with a stained nucleus and a faint acidophil cytoplasm, indicating a considerable reduction in the number of cell organization or specific structures within rats' bones. Some of the osteoblasts seemed empty as a result of osteocyte lysis, as seen.

In the current study, rats' ovariectomy resulted in a reduction in shaft cortical bone thickness and osteocyte count. Numerous osteoporotic cavities were also seen. The current study's findings are consistent with those of **(Chen *et al.*, 2019); (Moshtaghin *et al.*, 2020); (Tanideh *et al.*, 2021); (Alharbi *et al.*, 2021); and (Sakhel and Yahya, 2022),** who reported that histomorphometric and statistical results of the outer cortical bone of OVX rats revealed a significant decrease in the mean outer cortical bone thickness compared with CN-control rats. The femoral bone of OVX rats likewise had resorption gaps, an uneven surface with osteoclasts, and a decrease in cortical and trabecular bone thickness.

 **(NC-)Negative control: bone is displaying normal cortical bone and normal trabecular bone with normal osteoid matrix and normal lacunae (arrow). (HE, 100X)**	 **(PC+) Positive control: Bone section of control positive animal showing short, thin and separated spicules of trabecular bone and displaying marked thinning of cortical bone (arrow). (HE, 100X)**
 **5%LS: Bone is show displaying loss of chon droid matrix and little damaged osteoblasts and lacunae (HE, 100X)**	 **10%LS: Bone is displaying marked repairing of cortical bone (arrow). (HE, 100x)**
 **10%FS: Bone is displaying reforming of osteoid matrix and osteoblasts in the cortical bone (arrow). (HE, 100x)**	 **15%FS: displaying a normal cartilaginous layer and a discernible increase in trabecular bone thickness and density. (arrow). (HE, 100X)**
 **5%LS+10%FS: Normal lacunae, osteoid matrix, and cortical and trabecular bone are all visible in bone. (arrow). (HE, 100X)**	 **10%LS+15%FS: bone exhibits normal lacunae, normal osteoid matrix, and normal cortical and trabecular bone. (arrow). (HE, 100X)**

(Pict 3): Histopathological examination of femur bone.

As a conclusion the oral administration of OVX rats with phytoestrogens such as Lepidium Sativum and flaxseed was found to ameliorate histological and histochemical alterations, as well as morphometric parameters in bone with ovariectomy-induced osteoporosis.

Phytoestrogens appear to have a greatest promise for bone loss prevention and have received attention as a prospective agent in preventing and treating PMO, cancer prevention, and menopausal symptoms relief. Phytoestrogens resemble mammalian estrogens in both structure and action. There is evidence that diets high in phytoestrogenic isoflavones are connected with a lower risk of OP and menopausal symptoms.

DEXA scan:

Dual Energy X-ray Absorptiometry (DEXA) is widely regarded as the standard approach for diagnosing osteoporosis.

DEXA scan method is to evaluate BMD, bone mass (bone mineral content (BMC), muscle mass (lean), and fat by scanning femur bone samples with a DEXA scan machine at the National Research Center.

BMD and BMC may be examined within a particular range, and dual energy can be employed to eliminate measurement errors caused by soft tissue under measurement. DEXA studies are also beneficial for determining the impact of diet or medications on bone disorders, such as osteoporosis, by assessing BMD changes. Other advantages include cost effectiveness and reduced time consumption.

Table (8) summarizes the effects of Lepidium Sativum and Flaxseed on DEXA scans on the bones of femur rats with osteoporosis.

Results in table (8**)** revealed that, treating ovariectomized rats suffering from Osteoporosis with Lepidium Sativum and Flaxseed showed a significant increase on BMD, as compared to the Control Positive group which fed on basal diet only.

As a result on table (8) it could be observed that, the values of (BMD) in the Control Positive group decreased as compared with Control Negative which is 0.1454 for (PC+) vs 0.1872 for (NC-).

Data in table (8) revealed that, BMD was observed significant differed between the groups fed on diet Fortified with Lepidium Sativum and Flaxseed. The best results in BMD was recorded for the group treated with (10%LS+15%FS) which result about 0.1761 vs other groups compared with NC which is **0.1872**. It can be observed that the best group result is nearly to the negative control.

Our findings were consistent with those of **(Alshafei *et al.*, (2020); El-Haroun *et al.*, (2020); Mabrouk *et al.*, (2020); Sangondimath and Sen, (2023), and Garg *et al.*, (2024),** who realized that DEXA scan is the best way to diagnose osteoporosis, and that phytoestrogens such as Lepidium Sativum and flaxseed may improve bone with ovariectomy-induced osteoporosis.

Table (8): Effects of Lepidium Sativum and Flaxseed on DEXA scanned bone:

Region	BMD (g/cm)3	BMC (g)	Area (cm2)	Lean Mass (g)	Fat mass (g)
(NC-)Negative control	0.1872	0.8674	3.911	0.0174	0.7860
(PC+) Positive control	0.1454	0.4663	3.926	0.0186	0.9979
5%LS	0.1608	0.6008	3.436	0.0177	0.8225
10%LS	0.1711	0.7225	3.776	0.0179	0.8763
10%FS	0.1499	0.4988	3.852	0.0136	0.7814
15%FS	0.1555	0.5634	3.907	0.0188	0.8234
5%LS+10%FS	0.1607	0.6700	3.832	0.0201	0.8113
10%LS+15%FS	0.1761	0.7985	3.845	0.0288	0.8072

Bone Mineral Content **(BMC)**: represents the weight of all the bones in your body measured in grams (g).

Sensory evaluation of energy bars supplemented with different levels of Lepidium Sativum LS, Flaxseed FS seeds and their combination.

The average scores obtained by the energy bars product in the sensory evaluation are presented in Table (9). Revealed that, color in all supplemented energy bars with different levels of Lepidium Sativum, Flaxseed and their combination decreased significantly ($p \leq 0.05$) in scores of colors, as compared with the control un supplemented energy bars because the energy bars samples became darker. Also, data showed a significant decrease ($p \leq 0.05$) in the odor of the energy bars supplemented with different levels of Lepidium Sativum, Flaxseed and their combination as compared with control. The mean values of the texture of supplemented energy bars showed, non - significant differences ($p \leq 0.05$), between supplemented energy bars as compared with control. While the general acceptance of energy bars supplemented with (15% FS), (5% LS+ 10%FS), (10%LS+15% FS) was the most liked among all the judges. The lowest score of general acceptance was recorded for the energy bars supplemented with (10 % LS). On the other hand, data showed that the mean values of total scores decreased gradually with increasing the level of Lepidium Sativum, Flaxseed, and their combination. The results of the sensory evaluation showed an acceptable energy bar prepared in most proportions of the study.

Table (9): Sensory evaluation of energy bars supplemented with different levels of Lepidium Sativum LS, Flaxseed FS seeds and their combination.

Treatment	Color (20)	Odor (20)	Texture (20)	Taste (20)	General acceptable (20)	Total Score (100)
control	19.30 ± 0.67 [a]	19.60 ± 0.51 [a]	19.30 ±0.94[a]	19.60 ±0.51[a]	19.30 ± 0.67[a]	97.10 ± 2.37[a]
(5 % LS)	17.90 ± 3.03 [bc]	17.20 ± 3.45 [b]	17.70 ±2.86[abc]	16.40 ±3.68[bc]	18.60 ± 2.50[bc]	87.80 ±12.85[c]
(10 % LS)	17.20 ± 3.35 [c]	16.20 ± 3.70 [b]	16.20 ±3.48[cd]	16.95 ±3.87[bc]	16.10 ± 3.57[d]	83.25 ± 17.70[c]
(10% FS)	18.20 ± 2.78 [bc]	18.60 ± 2.22 [b]	18.42 ±3.11[bcd]	18.65 ±2.80[b]	18.85 ± 2.35[bc]	92.72 ± 11.32[b]
(15% FS)	18.75 ± 2.07 [b]	18.35 ± 3.44 [b]	19.05 ±2.98[ab]	18.75 ±3.45[b]	19.38 ± 1.80[ab]	94.28 ± 12.06[b]
(5%LS+10%FS)	17.05 ± 2.60 [bc]	18.20 ± 2.44 [b]	17.94 ±2.89[bcd]	17.10 ±3.03[bc]	19.23 ± 2.32[ab]	90.52 ±10.51[bc]
(10%LS+15%FS)	18.07 ± 2.60 [bc]	18.60 ± 2.22 [b]	17.80 ±4.02[d]	17.40 ±3.65[bc]	18.90 ± 3.90 [ab]	90.07 ± 15.69[bc]

(NC) Negative control**, (PC)** Positive control**, (LS)** Lepidium Sativum**, (FS)** Flaxseed

conclusion

As conclusion the results showed that Lepidium Sativum and flaxseed seeds are rich in minerals and a range of effective preventive compounds that effectively improved hormone levels and improved indicators of osteoporosis in the bones. Flaxseed and Lepidium Sativum seeds can be used as functional foods in a variety of products to lower the risk of osteoporosis. Lepidium Sativum and flaxseed seeds can eventually be added to functional meals in amounts of up to 15%.

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الملخص العربي

دور البذور كمواد مغذية ضد اختلال التوازن الهرموني وهشاشة العظام في الفئران

يعد نقص الهرمونات لدى النساء بعد انقطاع الطمث أحد عوامل الخطر الرئيسية لهشاشة العظام. كان الغرض من هذة الدراسة هو تقييم تاثير الوجبات المدعمة بحب الرشاد وبذور الكتان على الهرمونات وهشاشة العظام فى فئران التجارب مستأصلة المبيض .تضمنت الدراسة جزأين، **الجزء الأول:** التقييم البيولوجي حيث تم تقسيم ٤٠ فأراً إلى ثماني مجموعات متساوية، وزن كل منها يتراوح بين ٢٠٠±١٠ جرام. المجموعة (١) كانت كمجموعة ضابطة سالبة ، بينما خضعت السبع مجموعات لإستئصال المبيض ، وتم تغذية المجموعة (٢) على الوجبة الاساسية كمجموعة ضابطة موجبة.كما تم تغذية المجموعة (٣) على الوجبة المدعمة بالبذوربالنسب المختلفة لمدة ثمانية أسابيع. تمت مراقبة أوزان الفئران أثناء التغذية، وتم أخذ عينات البول لتقدير مستويات الكالسيوم والفوسفور، وتم جمع عينات الدم لتقييم مستويات هرمون الاستروجين والبروجستيرون وهرمون الغده الدرقية والكالسيوم والفوسفور والفوسفاتيز القلوي العظمي والأوستيوكالسين. تم تشريح وتحليل عظام الفخذ للأغراض النسيجية وفحص الديكسا . أظهرت النتائج أن لحب الرشاد وبذور الكتان تأثير مضاد لهشاشة العضام وتحسيين مستوى الهرمونات فى الفئران مستأصلة المبيض. حيث أظهرت تحسنا نسبيا فى وزن الجسم والرحم إلى جانب زيادة فى مستوى هرمون الأستروجين والبروجسترون وتحسين مستوى هرمون الغدة الدرقية ،وتحسين مستويات الكالسيوم والفسفور ، إنزيم الفوسفاتيز العظمي القلوي والأوستيوكالسين . إلى جانب إنخفاض إفراز الكالسيوم والفسفور فى البول . وكشف الفحص النسيجي وفحص الديكسا عن زيادة معنوية ملحوظة فى كثافة عظام الفخذ. **الجزء الثاني:**التقييم الحسي وقد تضمن تحضير سبعة عينات من منتج غذائي (ألواح الطاقة) بنسب مختلفة من حب الرشاد وبذور الكتان.وقد أشارت نتائج التقييم الحسي الى أن جميع العينات كانت مقبولة حسيا. توصي الدراسة بضرورة تدعيم وجبات السيدات بعد انقطاع الطمث بحب الرشاد وبذورالكتان لعلاج الخلل الهرموني وعلاج هشاشة العظام لدى النساء بعد انقطاع الطمث.

الكلمات المفتاحية: حب الرشاد؛ بذور الكتان؛ الاستروجين ؛ إنقطاع الطمث ؛ هشاشة العظام.