

Protective Effect of *Lepidium Sativum* Seeds and Buttermilk on Osteoporosis in Female Rats

Rehab M. Morad

Nutrition and Food Science, Home Economics Dep., Fac. of Specific Education - Fayoum University

Sahar S. A. Soltan

Nutrition and Food Science, Home Economics Dep., Fac. of Specific Education - Fayoum University

Emily T. Hanna

Nutrition Biochemistry and Metabolism Dep., National Nutrition Institute

Dalia R. Hassan

Nutrition and Food Science, Home Economics Dep., Fac. of Specific Education - Fayoum University

Abstract

Glucocorticoid-induced osteoporosis (GIO) is one of the serious side effects which have become the most common secondary osteoporosis. The study aimed to evaluate the effect of different levels of *Lepidium sativum* seeds and buttermilk on glucocorticoid-induced osteoporosis in rats. **Methods:** The present study was carried out in the National Nutrition Institute NNI Cairo, on forty-eight female rats. The rats were divided into eight groups (six rats each). The first group was fed on a basal diet and represents the negative control, while the other seven groups were injected subcutaneously with betamethasone at a dose of 4 mg/ kg BW three times a week. One group of them represents the positive control. The other six groups were fed on a basal diet containing 10% and 20% *Lepidium sativum* seeds (LSs), 10% and 20% buttermilk (BM), 10% and 20% mixture of LSs and BM for a period of eight weeks. **Results:** The positive control group showed a significant decrease in serum Estradiol (E2), Calcium (Ca), Phosphorus (P) levels and Ca and P in femur bone and a significant decrease in total Bone Mineral Density

(BMD), and a significant increase in serum Alkaline phosphatase (ALP). On the other hand, all osteoporosis groups administrated with different levels of LSs and BM (10 and 20%) had a significant decrease in (ALP) and a significant increase in Ca, P in serum and Ca, P in bone and BMD, compared with the positive control group. The pathological examination of bone confirmed these results. **Conclusion:** Lepidium sativum seeds and buttermilk showed bone protection against glucocorticoid-induced osteoporosis in rats. Lepidium sativum seeds had a potent protective effect more than Buttermilk due to its content of isoflavones.

Key words: Osteoporosis, Lepidium Sativum, Buttermilk, phytoestrogen, Probiotic.

Introduction

Osteoporosis is a worldwide disease characterized by reduction of bone mass and alteration of bone architecture resulting in increased bone fragility and increased fracture risk. The prevalence of osteoporosis is expected to increase significantly in the future because of aging of the population (**Qassem et al., 2017**). The bone is partially made up of osteoblasts, which make bone. The bone matrix is comprised of osteoid, and the bone also contains osteoclasts which destroy or resorption bone. The activity of bone cells is determined by the balance of cytokines- imbalances result in osteoporosis (**Orr., 2019**).

According to the WHO, normal BMD is known as a T-score over 1 standard deviations (SD), osteopenia is known as a T-score between -1.0 and -2.5 SD, and osteoporosis is known as a T-score less than -2.5 SD (**Akkawi and Zmmerly., 2018**).

Glucocorticoids are widely used to suppress inflammation or the immune system. High doses and long-term use of glucocorticoids lead to an important and common iatrogenic complication, glucocorticoid-induced osteoporosis, in a substantial proportion of patients (**Chotiyarnwong and McCloskey., 2020**).

The prevalence of osteoporosis in Egypt was higher in men more than women who recorded 28.4% and 21.9% of men and women respectively (**Taha., 2011**).

Lepidium sativum belongs to The Brassicaceae family, It is a fast-growing edible herb that has been cultivated as a culinary vegetable throughout North America, Europe, and Asia. It is known as rashad or thufa in Saudi Arabia and is grown in different locations of the country sometimes known as Garden cress. (**Prajapati and Dave., 2018 and Alqahtani et al., 2019**).

The medicinal properties of *Lepidium Sativum* are due to its high concentration of phytochemicals. Lepidine, sinapic acid ethyl ester, non-dibenzylthiourea, non-dibenzylurea, and lepidimoide are active components found in the seeds. Glucotropaeolin and 2-phenyl ethyl glucosinolates were found in studies, as well as phenolic compounds, alkaloids, steroids, flavonoids, glycosides, and glucosinolates. Glucotropaeloin, sinapin, sinapic acid, mucillagenous substance, and uric acid are all alkaloids found in the seeds (**Jain and Grover., 2018**).

Lepidium sativum seeds have traditionally been used to heal fractures and injuries. To mend fractures and internal injuries, seeds are combined with water or powdered and applied to damaged regions or ingested with water or warm milk in some parts of India. (**Prajapati and Dave., 2018**).

Buttermilk is sometimes associated or even confused with sour milk, natural (traditional) buttermilk, cultured milk, cultured buttermilk and cultured skim milk or even sometimes with fermented milk. Buttermilk is a liquid produced when cream is churned into butter. Milk protein, lactose, and minerals are all water-soluble components of buttermilk (**Barukcic et al., 2019**).

In Upper Egypt, milk is poured into skin bags (Kerba) and left sour for periods determined by experience. Air is blown into the kerba before closing it tightly and shaking until the fat globules

coalesce. After the removal of butter the remainder is called laban khad or sour buttermilk (**Ahmed et al., 2018**).

The health benefits of buttermilk have been evidenced by many studies. Clinical trials studying the effects of buttermilk on various diseases (e.g., cholesterol reduction, blood pressure reduction, antiviral effects, and anticancer) have shown positive effects (**Kumar and Garsa., 2015**). Fermented milk has various health benefits because very healthy bacteria are being prevalent in them termed as “probiotics” (**Hati et al., 2019**).

The objective of this study was to investigate the protective effect of *Lepidium Sativum* Seeds and Buttermilk at different levels on glucocorticoid- induced osteoporosis in female rats and their effect on liver and bone tissues.

Materials and Methods.

Materials:

Lepidium sativum (Garden cress) seeds were purchased from the local market, Fayoum, Egypt, and ground to obtain a powder. Fresh Buttermilk was purchased from farmer at the local market. The Kits used in the determination of Estradiol hormone, Serum Total Calcium, Serum Ionized calcium, Serum Alkaline Phosphatase and Serum Phosphorus were purchased from Gama Trade Company. The betamethasone (Dexaglobe Ampoules) was purchased from Pharmacy.

Rats: Adult non-pregnant female forty-eight albino rats (aged 6 to 8 weeks and about 180 to 200g body weight) were obtained from the animal house of Helwan.

Methods:

Buttermilk was placed in a closed cloth to get rid of excess whey for about two hours at room temperature for drainage. Then, it was placed on trays and dried in the sun for 8-10 hours (**Tamime and O'connor 1995 and Hassan., 1997**).

The chemical composition (protein, fat, moisture and carbohydrates calculated by difference) was determined according to the method of (A.O.A.C. 2007). Calcium was measured by Flame Photometer using the calcium hydroxide band emission at 622nm. However, the main atomic emission occurs at 423 nm. Phosphorus was determined according to the method of (Pulliainen and Wallin., 1996). Determination of isoflavones (Isorhamnetin, Daidazein, Genistein, Isoformentine, Biochainin) was done by HPLC according to (Coward et al., 1993).

Biological study:

Diet: Standard diet was prepared from fine ingredients per 100 gm diet according to Reeves et al. (1993). The diet had the following composition: corn oil 10%, salt mixture 4% according to (Hegsted et al.,1941), vitamins mixture 1% according to A.O.A.C. (1990), cellulose 5%, DLMethionin 0.3% and coline chloride 0.2 %, Casein protein was added at 14% level at the expense of corn starch up to 100 gm.

The experiment was done in the animal house of the National Nutrition Institute. Rats were housed individually in wire cages and were fed on a basal diet for one week to adapt.

After 1 week of adaptation, 48 rats were divided into eight groups (six rats each). Rats were matched for average body weight and housed individually in wire cages. Feed and water were provided ad libitum. The first group was fed on a standard diet and represented the negative control, while the other seven groups were injected subcutaneously with betamethasone at a dose of 4 mg/ kg BW three times a week for eight weeks. One group of them was fed on standard diet and represents the positive control. The other six groups were fed on a standard diet containing 10%, 20% Lepidium sativum seeds (LSs) and 10%, 20% dried buttermilk (BM) and 10% of the mix (5% LSs + 5% BM) and 20% of mix (10% LS +10% BM) for a period of eight weeks. During the experimental period rats weight and feed consumption were recorded twice weekly, to determine feed intake and body weight gain%, according to (Chapman et al.,1959).

Biochemical analysis:

At the end of the experimental period (8 weeks) the rats were sacrificed under anesthesia and blood samples were drawn from hepatic portal vein in centrifuge tubes to get the serum. Blood samples were collected into plain tubes without anticoagulants and allowed to clot. Blood samples were centrifuged at 3000 rpm for 10 min at room temperature, to obtain clear serum. Serum was frozen at -18°C until analyzed. The organs (liver, kidney, and femur bone) were separated from each rat then cleaned, washed in saline solution, dried with filter paper and weighed. Then they were kept in formalin solution 10% until histological investigations. The left femurs were weighted and put in foil paper and kept in a deep freezer until analyzed.

The separated serum was used for the determination of calcium and phosphorus according to the method of (Burtis et al., 2012), alkaline phosphatase (ALP) according to the method by (Bergmeyer et al., 1986), estradiol by enzyme-linked immune sorbent assay (ELISA) according to (Saldanha et al., 2011).

Bone analysis:

Determination of Bone Mineral Density (BMD) and Bone Mineral Content (BMC) were measured in the right femur of each animal by Dual Energy X-Ray Absorptiometry (DEXA) using Norland XR 46, version 3.9.6/2.3.1 instrument equipped with dedicated software for small animal measurements in bone mineral density unit, Medical Service Unit, National Research Center, Dokki, Egypt., this technique provides a software measure of right femur proximal, middle, distal and total area.

Determination of Calcium and Phosphorus in the left femur was done according to (Qin et al., 2021). The bone powder (~ ,1 g) was accurately weighed and put into digestion tubes. The samples were preliminarily digested for 1 h with 6 mL of HNO_3 (68%). Then, 2 mL of H_2O (30%) were added into tubes and microwave digestion was carried on via a microwave digestion instrument (Multiwave Go plus Anton paar) Table (A). Determination was done by (Agilent technologies 4210 MP-AES) and conditions were shown in Table (B).

Table (A)

Recipe	Note	Parameters	Program			
Rotors 24HVT50 24HVT80 41HVT56						
0.1-.05g sample Reagents: *3-6ml HNO ₃ *0-1 ml H ₂ O	If is HCL required (e.g. for stabilization of elements) add 0.5-1 ml HCL after digestion (prior to dilution) to the vessel and mix thoroughly.	Internal temperature limit: 200c Temperature control mode: AVG	Temperature program			
			Ramp	Temp	Hold	Fan
			20.00	190 70	20.00	1 3

Table (B)

Parameter	Ca	P
Wave	714.815	213.618
Repeat	3	3
Pump speed	15rpm	15rpm
Up tare time	15 s	10s
Stabilization time	15s	10s
Calibration error	5%	3%
Number spiked	3	3
Nebulizer flow	0.6 L/min	0.35L/min

Histopathology evaluation:

At necropsy, the liver is fixed in 10% buffered formalin until analysis. Tissue slices were routinely processed for paraffin embedding and sections were prepared and stained with hematoxylin and eosin. Histopathological assessment was performed on all groups. Right femur specimens were fixed in 10 % neutral buffered formalin for 24 h, decalcified in 10 % EDTA solution (pH= 7.4) and then processed till embedding in paraffin. Thin paraffin sections (4 μ m) were stained with H&E (**Bancroft et al., 1996**).

Statistical analysis:

The results obtained were analyzed using SPSS program by the one-way analysis of variance (ANOVA), followed by least significant difference (L.S.D) test to compare between groups. Results were expressed as mean \pm standard deviation (SD) and values of $P > 0.05$ were considered non-significantly different, while those of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significant, highly and very highly significant, respectively (**Armitage and Berr., 1987**).

Results and Discussion.

Table (1) showed the chemical composition of LS seeds and BM. The higher value of protein, Ca and P were found in BM while the highest value of fat, fiber and carbohydrate were found in LS seeds. These findings agreed with (**Jain and Grover., 2018**) who reported that LS seeds contain (21% - 25%) protein and a good amount of fat (23 -25%) and (30 - 40%) carbohydrate, and (**Gokavi et al., 2004**) who reported that LS seeds have a higher content of calcium and phosphorus.

Data presented in Table (2) observed that the high value of phytoestrogens in *Lepidium Sativum* seeds especially isoflavones isorhamtine, daidzein, genistein, isoformentine and biochanin.

The isoflavones genistein and daiszein are among the most active phytoestrogens (Hloucalova et al., 2016).

Results obtained by HPLC for Isoflavonoids were harmony with the results of (Nathawat et al., 2015) who found that Formononetin (Biochanin), Genistein and Daidazein were isolated from Lepidium Sativum seeds samples.

Table (1): The chemical composition of lepidium sativum and buttermilk (on dry weight basis).

Parameters	Unit	Lepidium sativum	Buttermilk
Protein	g/100g	22.4	48.7
Fat		22.0	7.0
Ash		5.5	9.3
Moisture		6.46	6.85
Fiber		10.79	-
Carbohydrate (by difference)		32.85	28.15
Ca	mg/100g	243.7	1135
P		590.7	950

Table (2): Isoflavonoids analysis of lepidium sativum seeds.

Flavonoids	Concentration (ppm)
Isorhamtine	451.7
Daidazein	57.36
Genistein	62.03
Isoformentine	1140
Biochainin	28.45

Data in Table (3) illustrated the effect of LS seeds and BM on total and ionized calcium and phosphorus levels in serum. There is a significant decrease $p < 0.05$ in serum calcium and phosphorus concentration in the positive control group. The decrease in calcium

may be due to cortisone leading to decrease calcium absorption from the intestinal (**Soltan, 2013**).

While there is a significant increase ($p < 0.05$) in the concentration of total calcium in the serum of all the experimental groups, but there was a non-significant different ($p > 0.05$) in concentration of calcium in 10% LS seeds and 10% BM groups, these results may be due to that lepidium sativum seeds and buttermilk were good source of calcium and easily absorbed and these results were in harmony with the results of estradiol. (**Nie et al., 2020**) demonstrated that estrogen is involved in the regulation of intestinal calcium absorption and presence of estrogen receptors in the intestine has been reported and has been shown to increase intestinal calcium absorption in both rats and humans.

Female rats treated on LS seeds showed a highly significant level in serum phosphorus at ($p < 0.05$) when compared with the positive control group. These results may be due to that LS seeds are a good source of phosphorus as the study reported by (**Abd El-Salam et al., 2019**) who found that LS seeds contain high levels of potassium and phosphorus. Also (**Dixit Jr Lii et al., 2020**) reported that the rise in serum phosphorus is due to increased osteoblastic activity.

The present results in the Table (4) showed that 8 weeks of BM and Lepidium sativum seeds significant increased ($p < 0.05$) in the level of E2 in serum female rats compared to their positive control group, special 20%LS seeds, 20%BM, 10%Mix, 20%Mix groups high significant increase in the level E2, but it was not significant ($p > 0.05$) different in 10% LS seeds and 10%BM. (**Lee et al., 2021**) indicated the positive role of fermented milk on serum Estradiol level. Despite the lacks of research on this matter, The positive role of LS seeds on serum Estradiol level is attributed to the phytoestrogens constituent in the seeds, since Phytoestrogens (plant estrogens) are substances that occur naturally in plants, They have a similar chemical structure to our own body's estrogen (one of the main female hormones). Molecular mechanisms of action,

phytoestrogens are from one to four orders weaker ligands of the estrogen receptors (ERs) than 17β -estradiol. Intake of phytoestrogen, can reach tens to hundreds of milligrams per day, that shown to be more effective concentrations in bodily fluids. Isoflavones have a several times higher affinity for $ER\beta$ than for $ER\alpha$, and thus act as partially selective modulators of ERs (Kolatorova et al., 2018). Our results revealed a decreased level of ALP in all treated rats compared to their positive control group. LS seeds and BM significantly improved serum ALP level after 8- week administration, suggesting that the plant can activate the osteoblast differentiation and bone formation; meanwhile, it can inhibit osteoclast function and bone resorption (Abdel-Kader et al., 2017).

Table (3): Effect of lepidium sativum seeds and buttermilk on calcium and phosphorus levels in serum of osteoporotic rats.

Parameters	Total Ca (mmol/l)	Ionized Ca (mmol/l)	Phosphorus (mmol/l)
Negative control group	2.70±0.97	1.14±0.39	6.48±1.67
Positive control group	1.28±0.20 ^a	0.51±0.15 ^a	1.87±0.38 ^a
10% lepidium sativum seeds group	2.36±0.14	1.12±0.26 ^b	4.87±0.60 ^b
20% lepidium sativum seeds group	3.42±0.33 ^b	1.43±0.33 ^b	4.76±1.09 ^b
10% Buttermilk group	2.04±0.45	0.89±0.26	3.57±0.68 ^a
20% Buttermilk group	3.07±0.85 ^b	1.39±0.36 ^b	2.229±0.70 ^a
10% Mix of (BM&LSs)	2.92±0.34 ^b	1.37±0.19 ^b	3.05±0.75 ^a
20% Mix of (BM&LSs)	2.65±0.37 ^b	1.181±0.23 ^b	2.108±0.87 ^a

a: significant difference with negative control, **b:** significant difference with positive control.

Table (4): Effect of lepidium sativum seeds and buttermilk on Estradiol hormone and Alkaline phosphatase enzyme.

Parameters Groups	ALP(U/L) Alkaline phosphatase	E2 (pg/ml) Estradiol
Negative control group	59.50±9.73	37.57±14.62
Positive control group	128.80±36.81 ^a	13.40±2.56
10% lepidium sativum seeds group	76.20±10.40 ^b	38.30±9.92
20% lepidium sativum seeds group	68.00±15.91 ^b	47.60±7.82 ^b
10% Buttermilk group	61.50±9.40 ^b	39.97±7.63
20% Buttermilk group	71.40±10.43 ^b	51.50±6.37 ^b
10% Mix of (BM & LS)	66.50±11.29 ^b	57.30±11.71 ^b
20% Mix of (BM & LS)	73.26±25.88 ^b	47.73±13.34 ^b

a: significant difference with negative control, **b:** significant difference with positive control Values are expressed as mean ±SD. Significant at p<0.05 using one way ANOVA test.

Data present in Table (5) showed the effect of LS seeds and BM on Bone Mineral Density (BMD), 20% LS seeds, 20% BM, 10% Mix and 20% Mix groups were significantly higher (p<0.05) as compared with the positive control group which cleared, that osteoporosis caused a significant decrease of BMD of the femur bone. The Glucocorticoid has deleterious effect on bone density, and led to suppression of bone formation by a decreasing the number and functioning of osteoblast and induced bone loss (**Sarkis et al., 2012 and Okafor et al., 2016**).

Table (5): Effect of lepidium sativum seeds and buttermilk on bone mineral density (BMD) and bone mineral content (BMC) of osteoporotic female rats

Parameters Groups	BMD g/cm ²	BMC g/cm ²
Negative control group	0.16±0.01	0.015 ±0.01
Positive control group	0.09±0.03 ^a	0.011±0.01
10% lepidium sativum seeds group	0.12±0.01	0.014±0.01
20% lepidium sativum seeds group	0.13±0.158	0.015±0.00
10% Buttermilk group	0.14±0.01	0.015±0.01
20% Buttermilk group	0.16±0.04 ^b	0.015±0.01
10% Mix of (BM&LS)	0.17±0.03 ^b	0.013±0.00
20% Mix of (BM&LS)	0.15±0.04 ^b	0.015±0.01

a: significant difference with negative control, **b:** significant difference with positive control Values are expressed as mean ±SD. Significant at p<0.05 using one way ANOVA test.

Data in Table (6) illustrated a significant increase (p<0.05) in calcium and phosphorus femur bone in all experimental groups. The mean value ±SD of femur bone calcium and phosphorus of positive control group decrease significant (p<0.05), as compared to the negative control group, these results were in agreement with (**Lin et al., 2014 and Banji et al.,2014**) who reported that levels of bone Ca and P were decreased in the prednisolone acetate treated rats. This may be because prednisolone acetate enhances urinary excretion of Ca and P and reduces intestinal absorption.

The present study results showed a highly significant increase in femur bone calcium and phosphorus femur bone suggesting that Ca was utilized effectively in the remineralization process of bone. (Alharbi et al., 2021) showed the LS seeds high content of unsaturated fatty acids which exert their biological activity in improving the osteogenic markers that enhance bone healing. Thent et al., 2019 reported that phytosterols and phytoestrogens are some important compounds present in higher quantities in LS plant. Phytoestrogens involved in bone metabolism, and can be useful for the prevention and treatment of many diseases including osteoporosis.

Table (6): Effect of lepidium sativum seeds and buttermilk on calcium and phosphorus in bone (femur) of osteoporotic female rats.

Parameters Groups	Calcium (mg/g)	Phosphorus (mg/g)
Negative control group	23.00±1.02	10.683±3.44
Positive control group	11.14±2.65 ^a	6.70±0.83 ^a
10% lepidium sativum seeds group	15.80±1.50 ^{a,b}	9.36±0.33 ^{a,b}
20% lepidium sativum seeds group	18.82±0.34 ^{a,b}	9.30±0.32 ^{a,b}
10% Buttermilk group	15.49±0.31 ^{a,b}	8.10±0.43 ^{a,b}
20% Buttermilk group	17.08±2.57 ^{a,b}	9.36±0.33 ^{a,b}
10% Mix of (BM&LS)	16.00±1.22 ^{a,b}	8.60±0.23 ^{a,b}
20% Mix of (BM&LS)	15.51±0.60 ^{a,b}	7.70±0.311 ^{a,b}

a: significant difference with negative control, **b:** significant difference with positive control.

The results for the present study are similar with the results of the previous studies. (Dixit Jr Lii et al., 2020) reported that glycosides, mucilage and other phenolic compounds promote in callus formation, the flavonoids and fatty acids and protein found in *Lepidium sativum* seeds that have aided in bone rebuilding, and the present study proposed the same mechanism.

Also, (Rizzoli and Biver., 2018) showed that fermented milk products, such as yoghurt, promotes bone growth and homeostasis through a variety of mechanisms involving important minerals like calcium, phosphorus, and protein, as well as pre- and probiotics that alter gut microbiota composition and metabolism .Although it's still unclear whether fermented milk products are a cause or simply a marker of a healthy lifestyle that promotes bone health.

Histopathological results:

Microscopically, the liver of the negative control rat showed normal histological structure of central veins, portal areas (arrow) and hepatin parenchymal cells (Fig.1). Section from the liver of Betamthasone administrated rat (positive control) showing marked priportal vacuolar degeneration of the hepatic cells of the micro (short arrow) and necrotic cells (thin arrow) (Fig. 2). These results are in agreement with (Soltan, 2013 and Mohamed et al., 2021) who reported the liver of rats treated with synthetic corticosteroids causes histologic abnormality changes in the liver and necrosis of sporadic hepatocytes.

While, the section of the liver of Betamethasone administrated female rat and treated with 10% LS seeds showing moderate degree of hepatocellular vacuolar degeneration and necrosis with scattered fat cysts, (arrow) (Fig.3). Meanwhile liver of Betamethasone administrated rat and treated with 20% LS seeds showing a good degree of protection of the hepatic cells with mild degenerative and necrotic changes (Fig.4). These results are in agreement with (Althnaian., 2014) who reported that a higher dose of *Lepidium sativum* (6 g/kg diet) is safe for liver function in rats.

(Jain and Gover., 2018 and Alharbi et al., 2021) reported that LS seeds high content of unsaturated fatty acids a special linolenic acid.

The section liver of Betamethasone administrated female rat and treated with 10% BM showing the moderate degree of hepatic steatosis, many fat cyts (Fig. 5). While, liver of Betamethasone administrated female rat and treated with 20% BM showing good protection of the hepatic parenchymal cells with only a few scattered degenerated and necrotic (arrow) cells (Fig. 6). (Rayes et al., 2008) who reported that fermented milk was fed to rats for four weeks showed a structure of a healthy and normal liver structure similar to that of controls.

Moreover, of liver of Betamethasone administrated female rat and treated with 10% Mix showing mild micovesicular vacuolar degeneration (arrow) and scattered necrotic cells (Fig. 7). Also, liver of Betamethasone administrated rat and treated with 20% Mix showing moderate degree of vacuolar degeneration (arrow), scattered fat cells (short arrow) and necrosis (dotted arrow) (Fig. 8).

Microscopically, bone of rat negative control group fed on a commercial diet (Fig. 9), showing the normal histological structure of the periosteum, while in positive control group (Fig. 10) which fed on a commercial diet and were injected with betamethasone to cause osteoporosis showing osteoporosis and Longitudinal sections of femurs diaphysis compact bone rats showed decreased intensity of the bone matrix. these results agree with (Soltan, 2013 and Mohamed ., 2021) who proved that existence of osteoporosis and resorption or the bone trabeculae. While bone of Betamethasone administrated rats and treated with 10% LS seeds (Fig. 11) showed mild increased intensity of the bone matrix with still scattered small bone erosions full of osteoclasts and increased number of osteocytes, bone of Betamethasone administrated rats and treated with 20% LS seeds (Fig, 12) increase the matrix intensity with mineralization, few erosive areas and increased number and appearance of lines of bone deposition.

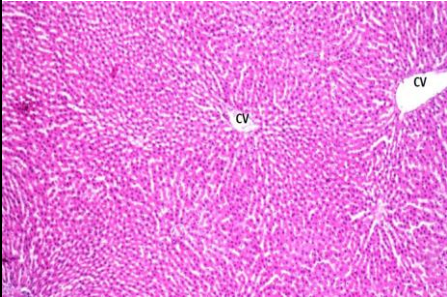
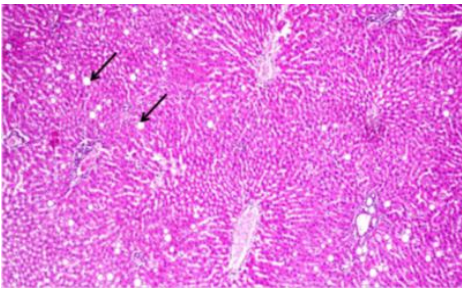
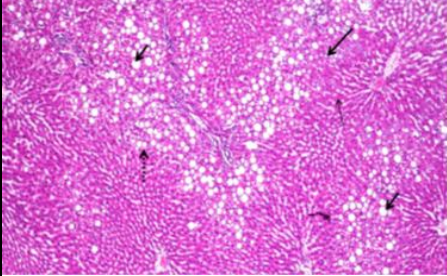
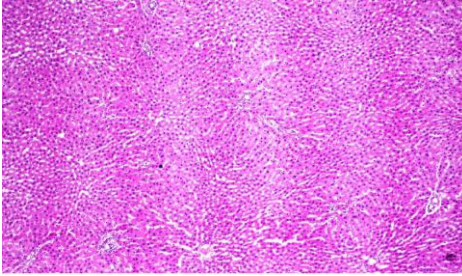
(Abdel-Kader et al., 2017) who proved that *L. sativum* extract rats revealed marked improvement as compared to those of the osteoporotic rats. The cortical bone thickness was very similar to the normal control group.

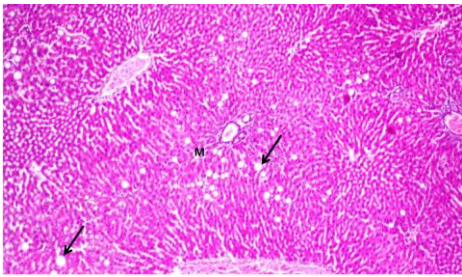
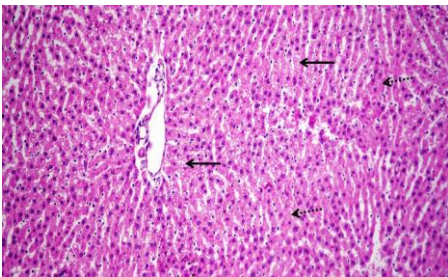
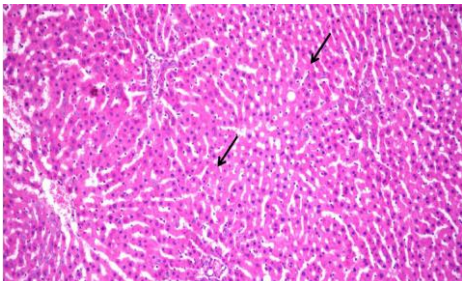
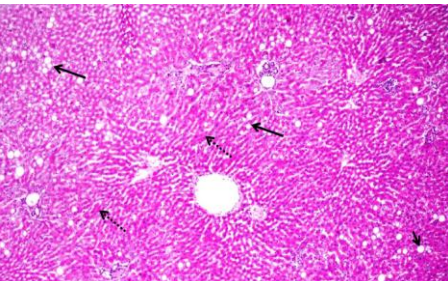
The bone of betamethasone administrated rats with 10% BM (Fig.13) showed areas of bone resorption, bone erosions, with sometimes layers of bone deposition, while treated with 20% BM (Fig. 14) showed layers of bone deposition, good appearance of bone matrix, very few erosive areas and many osteocytes.

(Lee et al., 2021) who reported that milk products fermented can exhibit anti-osteoporosis effects on post-menopausal osteoporosis via regulating the expression of bone- metabolism-related-markers.

The bone of Betamethasone administrated rats and treated with 10% Mix (Fig. 15) showed dark layers of bone deposition and osteocytes in their lacunae with scattered erosive areas with osteoclasts while bone of Betamethasone administrated rats and treated with 20% Mix (Fig. 16) showed dark lines of bone deposition and osteocytes in their lacunae with regularly and tightly arranged collagen fibers and mineralization.

Histopathological examination for liver and bone tissue.

<p><u>Liver:</u></p>  <p>Fig. 1: Liver of control rat showing the normal histological structure of the central veins (CV), portal areas (arrow) and hepatic parenchymal cells (HCs). (H&E, X100).</p>	 <p>Fig.3: Liver of Betameth administrated rat and treated with 10% LSs showing the moderate degree of hepatocellular vacuolar degeneration. (H&E, X100).</p>
 <p>Fig. 2: Liver of Betamethasone administrated rat showing marked periportal vacuolar degeneration of the hepatic cells of the micro (arrow). (H&E, X200).</p>	 <p>Fig. 4: Liver of Betamethasone administrated rat and treated with 20% LSs showing a good degree of protection of the hepatic cells with mild degenerative and necrotic changes. (H&E, X100).</p>

	
<p>Fig. 5: Liver of Betamethasone administrated rat and treated with 10% BM showing the moderate degree of hepatic steatosis, many fat cysts (arrow). (H&E, X100).</p>	<p>Fig. 7: Liver of Betamethasone administrated rat and treated with 10% Mix showing mild microvesicular vacuolar degeneration (H&E, X200).</p>
	
<p>Fig. 6: Liver of Betamethasone administrated rat and treated with 20% BM showing good protection of the hepatic parenchymal cells with only a few scattered degenerated and necrotic (arrow) cells. (H&E, X200).</p>	<p>Fig. 8: Liver of Betamethasone administrated rat and treated with 20% Mix showing the moderate degree of vacuolar degeneration (H&E, X100).</p>

Histopathological examination for bone tissue

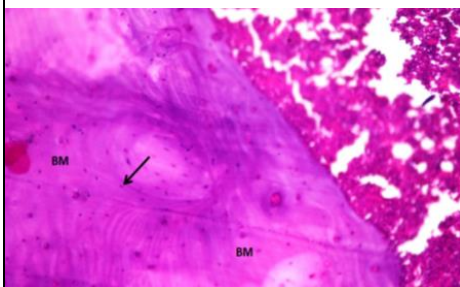


Fig.9: Longitudinal section of femur diaphysis compact bone of control rat showing normal bone architecture; osteocytes. (H&E, X200).

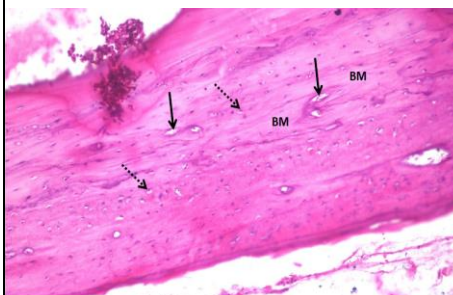


Fig.11: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 10% LSs showing mild increased intensity of the bone matrix (BM) (H&E, X100).

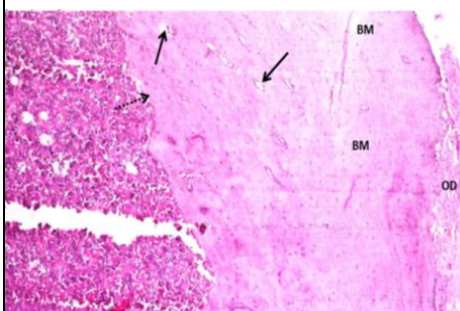


Fig. 10: Longitudinal section of femur diaphysis compact bone of Betamethasone administrated rat showing decreased intensity of the bone matrix (BM). (H&E, X100).

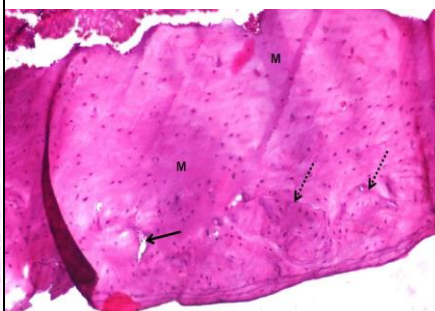


Fig. 12: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 20% LSs showing increased the matrix intensity with mineralization (M), (H&E, X200).

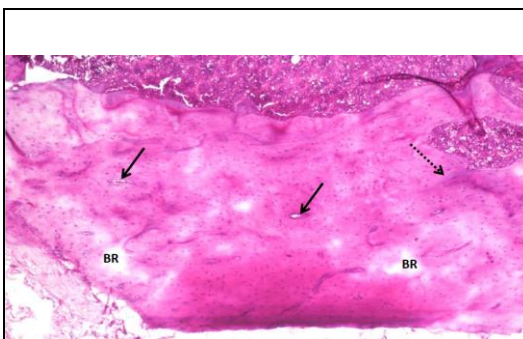


Fig. 13: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 10% BM showing areas of bone resorption (BR) (H&E, X100).

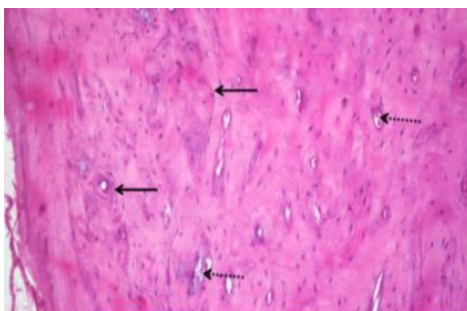


Fig 15: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 10% Mix showing dark layers of bone deposition and osteocytes in their lacunae with scattered erosive areas with osteoclasts (H&E,X200).

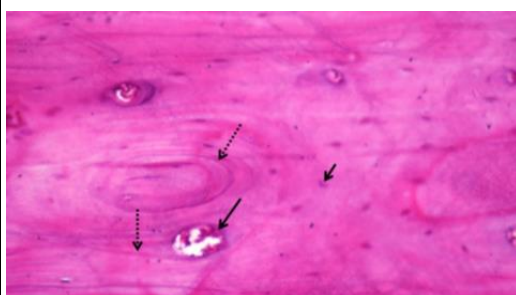


Fig. 14: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 20% BM showing layers of bone deposition (dotted arrow), good appearance of bone matrix (BM), (H&E, X400)

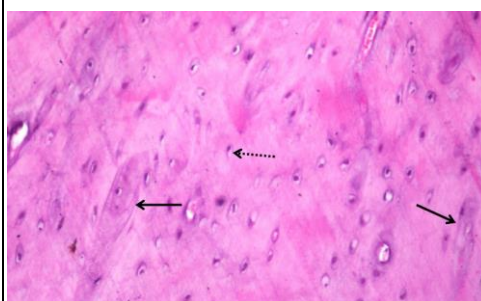


Fig.16: Longitudinal section of femur bone of Betamethasone administrated rat and treated with 20% Mix showing dark lines of bone deposition (arrow) and osteocytes in their lacunae (dotted arrow) (H&E, X400).

Conclusion:

In conclusion we have demonstrated that the protective effect of *Lepidium sativum* seeds and buttermilk through the duration of 8-weeks has significantly improved the biochemical analysis indices and restored and showed bone protection against glucocorticoid-induced in rats. These results may be due to the prebiotic effect and phytoestrogen and calcium and phosphorus in buttermilk and LS seeds This study may have implications for humans which indicates more researches into this important field of medicine. Long-term studies are needed to establish the potential public health benefit of LS and buttermilk with different levels by their incorporation by them in the diet.

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التأثير الوقائي لبذور حب الرشاد ومخيض اللبن علي مرض هشاشة
العظام لإناث الجرذان

رحاب محمد مرانة*، سحر سلطان عبد المجيدة، اميلي توفيق حنا*، داليا رفعت حسن*

*تغذية و علوم اطعمة - قسم اقتصاد منزلي- كلية تربية نوعية-جامعة الفيوم

*قسم كيمياء التغذية والتمثيل الغذائي المعهد القومي للتغذية

الملخص العربي

هشاشة العظام هي واحدة من الآثار الجانبية الخطيرة التي يسببها الجلوكوكورتيكويد حيث أصبحت أكثر أنواع هشاشة العظام الثانوية شيوعاً، تهدف الدراسة إلى: تقييم تأثير المستويات المختلفة من بذور حب الرشاد ومخيض اللبن على هشاشة العظام التي يسببها الجلوكوكورتيكويد في جرذان التجارب. الخطوات: أجريت الدراسة الحالية في المعهد القومي للتغذية بالقاهرة، مصر على ٤٨ من اناث الجرذان . تم تقسيم الجرذان إلى ثماني مجموعات (ستة جرذان لكل مجموعة). المجموعة الأولى تم تغذيتها على النظام الغذائي الاساسي وتمثل المجموعة الضابطة السالبة، بينما تم حقن المجموعات السبع الأخرى بالبيتاميثازون تحت الجلد بجرعة ٤ مجم / كجم من وزن الجسم ثلاث مرات اسبوعياً حيث مثلت مجموعة واحدة منهم المجموعة الضابطة الموجبة، تم تغذية المجموعات الستة الأخرى على نظام غذائي أساسي يحتوي على ١٠٪، ٢٠٪ بذور حب الرشاد و ١٠%، ٢٠% من مخيض اللبن و ١٠% مزيج من مخيض اللبن وبذور حب الرشاد لمدة ثمانية اسابيع. النتائج: أظهرت مجموعة البيتاميثازون إنخفاضاً معنوياً في مستويات E2,Ca,P في مصل الدم و Ca,P في عظم الفخذ و إنخفاضاً معنوياً في إجمالي كثافة العظم ، ومن ناحية أخرى ، أظهرت جميع مجموعات هشاشة العظام التي تناولت مستويات مختلفة من كل من بذور حب الرشاد و مخيض اللبن إنخفاضاً معنوياً في (ALP) وزيادة معنوية في Ca,P في مصل الدم و Ca, p في العظام و BMD ، مقارنة بالمجموعة الضابطة الموجبة وقد اكد الفحص الباثولوجي للعظام هذه النتائج . الخلاصة: أظهرت بذور حب الرشاد ومخيض اللبن حماية العظام ضد الجلوكوكورتيكويد علي جرذان التجارب. من النتائج التي توصلنا إليها، استنتجنا أن بذور حب الرشاد لها تأثير وقائي أكثر من مخيض اللبن بسبب احتوائها على الايزوفلافون والأحماض الدهنية.

الكلمات المفتاحية : هشاشة العظام – حب الرشاد – مخيض اللبن – فيتوايسترودجين- البروبايوتك.