Dietary Effect of Sesame Seeds and Soy Protein on Bone Density in Ovariectomized Rats

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التأثير الغذائي لبذور السمسم وبروتين الصويا على كثافة العظام في الفئران مستأصلة المبيض

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الملخص العربي :

هشاشة العظام هي واحدة من أكثر الامراض شيوعًا عند النساء في مرحله مابعد انقطاع الطمث ، حيث تؤثر على نوعية الحياة وتزيد من خطر الإصابة بالكسور. أجريت هذه الدراسة لتقييم تأثير الأنظمة الغذائية المدعومة ببذور السمسم وبروتين الصويا على هشاشة العظام في الفئران مستأصلة المبيض. تم اختيار أربعة وعشرين فأرا بصورة عشوائية وتقسيمهم إلى 4 مجموعات متساوية. المجموعة (1) المجموعة الضابطة السالبة وتغذت على النظام الغذائي الأساسي ، في حين أن المجموعات الثلاث الأخرى تم إخضاعهم لإستئصال المبيض. بعد ثلاثة أسابيع من استئصال المبيض ، تم تغذية المجموعة (2) على النظام الغذائي الأساسي كمجموعة ضابطة موجبة ، بينما تم تغذية المجموعتين (3) و (4) على أنظمة غذائية تجريبية مدعومة ب: (10٪ سمسم+ 50٪ بروتين صويا بدلاً من الكازين ، و 10٪ سمسم+100%بروتين صويا بدلاً من الكازين) على التوالي لمدة 8 أسابيع. تم تسجيل أوزان الجسم الأولية والنهائية .مع نهاية التجربة تم جمع عينات البول لتقدير مستوى الكالسيوم والفوسفوربالاضافة إلى جمع عينات الدم لتقدير مستويات الكالسيوم والفوسفور والفوسفاتيز القاعدي والأوستيوكالسن والإستروجين. تم وزن الرحم. كما تم تشريح عظام الفخذ للفحص النسيجي وأشعةDEXA . أدى إستئصال المبيض إلى إرتفاع مستويات الكالسيوم والفسفور والفوسفاتيز القاعدي والأستيوكالسن وانخفاض مستوى الأستروجين في سيرم الدم. وأظهرت المجموعات المعالجة بالوجبات المدعومة تحسنًا في جميع المؤشرات السابقة.الى جانب انخفاض مستويات الكالسيوم والفسفورفي البول.وظهور تحسن ملحوظ في كثافة عظام الفخذ في كل من الفحص النسيجي وأشعة DEXA .

الكلمات المفتاحية: هشاشة العظام المرتبطة بانقطاع الطمث، نقص هرمون المبيض، التحاليل البيوكيميائية، الفحص النسيجي للعظام ،أشعةDEXA.

Dietary Effect of Sesame Seeds and Soy Protein on Bone Density in Ovariectomized Rats

Abstract:

Osteoporosis is one of the most common disorders in postmenopausal women, affecting the quality of life and increasing the risk for fractures. This study was carried out to evaluate the effect of diets fortified with sesame seeds (SS) and Soy Protein (SP) on osteoporosis in ovariectomized rats (OVX). Twenty four rats were randomized into 4 equal groups. Group (1) was Control Negative and fed on basal diet, while the other 3 groups OVX-operated. Three weeks after ovariectomy, group (2) was fed on basal diet as a Control Positive, while groups (3) and (4) were fed on experimental diets fortified with the following: (10% SS+50% SP instead of casein, and 10% SS+100% SP instead of casein) respectively for 8weeks. During feeding period, initial and final body weights were recorded. At the end of the experiment, the last 24 hr. urine samples were collected for estimating Ca and P level. Blood samples were collected for estimating serum calcium (Ca), phosphorous (P), alkaline phosphatase (ALP), osteocalcin (OC) levels and estrogen. The uterus were dissected out and weighed. Femur bones also were dissected for Histological examination and DEXA scan. OVX cased a significant increase in the level of serum Ca, P, ALP, OC and decrease Estrogen level .treated OVX rats with fortified diets show an improvement in all previous parameters. Beside that decreased urinary excretion of Ca and P. There were also significant increases in femur bone density in histopathological examination and DEXA scan.

Keywords: Postmenopausal Osteoporosis; ovarian hormone deficiency; Biochemical analysis; histopathological examination and DEXA scan.

Introduction:

Bone is a living part of our body which grows. New bone cells grow throughout our lifetime and old bone cells break down to create room for the new, stronger bone. When there was osteoporosis the old bone broke down quicker than it can be replaced by the new bone. The bones loose minerals (such as calcium) as this happens. This makes the bones more fragile and more likely to crack even after a minor fracture, little bump or fall **(Howe *et al.*, 2011).**

Osteoporosis is often called a "silent disease" because initially bone loss occurs without symptoms. Osteoporosis is resulting from an imbalance in the bone remodeling process. The combination of over-functioning bone resorbing cells, osteoclasts, and insufficient functioning of bone forming cells, osteoblasts, eventually leads to the reduction of bone mass and the appearance of osteoporotic symptoms **(Sözen and Başaran, 2017).**

Millions of people around the world are suffering from osteoporosis, a condition marked by a loss of bone density and a weakening of bone cells that leads to fragility and leaves it sensitive to fractures, which in turn can lead to physical disabilities for the elderly **(Bartl and Bartl, 2019).**

Causes of osteoporosis factors include age rise, female sex especially at age 50 above, postmenopausal status, low bone mineral density (BMD), Deficiency of vitamin .medications **(Kovvuru *et al.*, 2020).**

Currently, osteoporosis is one of the most common problems, thus becoming a public health problem. Statistical studies have shown that over 50% of adults over age 50 suffer from osteoporosis, and of these about 70% are postmenopausal women **(Rizzoli, 2018).**

Women, after menopause, face undesirable conditions associated with decline of estrogens.

Postmenopausal osteoporosis is mainly caused by a sudden cessation of estrogen after the menopause **(Black and Rosen, 2016).**

Estrogen deficiency leads to series of metabolic alterations that break the balance between bone formation and bone reabsorption, and eventually result in a rapid reduction in bone mineral density **(Eastell and Szulc, 2017).**

The interest in the potential health effects of dietary phytoestrogens has increased with the findings that hormone replacement therapy is not as safe or effective as previously thought **(Domínguez-López *et al.*, 2020).**

The diagnosis of osteoporosis is determined by measuring BMD of the hip and spine using Dual Energy X-Ray Absorptiometry (DEXA scan),according to the guidelines of the world health organization, osteoporosis is classified as a BMD that lies below the average value of 2,5 standard deviation for healthy young women or more **(Jain and Vokes, 2017).**

Recent scientific advances have put additional light on the relationship between diet and human health. Nutrients play a significant role in avoid and prevention of many civilization diseases, such as osteoporosis, type II diabetes, hypercholesterolemia, and cardiovascular diseases **(Sathyapalan *et al.*, 2018)**

Sesamum indicum has strong biological and pharmacological properties such as contains lignan aglycones in oil and lignan. The seeds are a rich source of antioxidants and bioactive compounds. Sesame proteins consisted of lysine, tryptophan and methionine. Sesame oil is rich in linoleic and oleic acids, and high content of fat-soluble lignans (sesamin and sesamolin). Sesame seed contain vital minerals, vitamins, phytosterols, polyunsaturated fatty acids, Sesame seed is an excellent source of phosphorous, iron, magnesium calcium, manganese, copper and zinc **(Pathak *et al.*, 2014).**

Sesamin has inhibitory effects of on human osteoclast differentiation. The lignan effectively inhibits the human osteoclast differentiation process and consequently causes a decrease in resorption activity **(Wanachewin *et al.*, 2017).**

Soybean (Glycine max) is a species of legume native to East Asia, widely grown for its edible bean which has several uses. Soy is a good source of several vitamins and minerals, including vitamin K, folate, copper, manganese, phosphorus, and thiamine. It’s a rich source of various bioactive plant compounds **(Wijewardana *et al.*, 2019).**

Soy protein is considered to be a good substituent for animal protein and their nutritional profile except sulfur amino acids (methionine and cysteine) is almost similar to that of animal protein because soybean proteins contain most of the essential amino acids required for human nutrition **(Hassan, 2013).**Soy protein products such as flour, concentrates and isolates are used in the food industry because of their functionality, nutritional value and low cost. Soy and soy-based food represent the most important dietary source of phytoestrogens in humans **(Miniello *et al.*, 2013).**

Aim of study: This study is carried out to investigate the effect of Diets Fortified with Sesame Seeds and Soy Protein on Osteoporosis in Lab Rats.

Materials and Methods:

Materials:

Sesame seeds were obtained from the local market. Soy proteins isolate (protein about 85-90%) were obtained from Food Technology Research Institute (FTRI), Agricultural Research Center (ARC), Cairo, Egypt. Casein, all vitamins, minerals, cellulose, choline chloride were obtained from Elgomhoria company, Mansoura, Egypt.

Kits used to determine Serum Calcium (Ca), phosphorous (p), Bone Specific Alkaline Phosphatase (ALP), Osteocalcin (OC), urine Calcium, urine phosphorous, were obtained from Gamma tread Company (GTCO), Cairo, Egypt.

Animals:

Twenty four (24) normal female albino rats (Sprague Dawley Strain) weighing (200±10g) was be obtained from the laboratory animal colony. Nile Center Experimental Research, (NCER), Mansoura, Egypt.

Methods:

Chemical analysis:

Water, protein, fat, and ash, were determined as described in **(A. O. A. C. 2000).** While Total nitrogen extract due to fiber is carbohydrate were calculated by: The differences carbohydrates (%) = 100 – (water + fat + protein + fiber + ash).Fatty acid analysis: Total fatty acids were measured in SS and SP using a modification of AOAC official method **(AOAC, 1990).** Antioxidant capacity was determined by the method of **Gaoa *et al.*, (1998).**

Experimental design:

Twenty four (24) normal female albino rats (Sprague Dawley Strain) weighing (200±10g) were housed individually in metabolic cages under hygienic condition at room temperature of 25 ° C ,humidity of 50% and they housed 12 h light/12 h dark cycles. The rates were fed on basal diet one week and water was provided for adaptation .The experiment on rats was carried out according to the national regulation on animal welfare and animal committee.

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**(Campbel1.,1963)** , 3.5 % salt mixture **(Hegsted *et al.*, 1941)** and 5% fibers(cellules). The Remainder was corn starch up to 100 %. Experimental diets were formulated as follow:

1. Basal diet fortified with 10% ground sesame seeds and50% of soy protein instead of casein.
2. Basal diet fortified with 10% ground sesame seeds and100% of soy protein instead of casein.

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

Ingredients	Casein protein	sucrose	Corn oil	Choline chloride	Vit. Mix.	Salt mix.	cellules	Corn starch	Total amount
g	140	100	50	2.5	10	35	50	612.5	1000
%	14%	10%	5%	0.25%	1%	3.5%	5%	61.25%	100

After the period of adaptation on basal diet the rats were divided in to two main groups as follow:

The first main group (6rats): were fed on basal diet (as a control negative group)

The second main group (18 rats): were had an ovariectomy procedure and fed on basal diet for three weeks the duration of healing and to had estrogen deficiency to had osteoporosis .

Then the rats in this group were divided in to three subgroups (n=6).

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

Group NO	Diet and symbol	Description
Group 1	**(CN)**Control Negative	Fed on basal diet as Control Negative group
Group 2	(**CP**) Control Positive	Fed on basal diet as Control Positive group
Sub group 1	**G2**+10%SS+50%SP	Fed on basal diet containing 10%SS+50%SP
Sub group 2	**G2**+10%SS+100%SP	Fed on basal diet containing 10%SS+100%SP

(CN) Control Negative, **(CP)** Control Positive, **(SS)** Sesame seed**, (SP)** Soy protein

Ovariectomy operation in rats:

Anesthesia: ovariectomy in rats anesthesia with ketamine xylazine combination with 3:1 by injection I/p each rat with a dosage depending on its weight.

Operation: as illustrated in Photo (1), 3 cm longitudinal incisions in rat location of uterus just behind urinary bladder then locate each ovary and ligation of ovarian arteries and removal of ovary from abdominal cavity the suture muscle and skin individually with absorbable suture with simple continuous suture patterns Post-operative care with antibiotic and anti-inflammatory according to **(Lasota and Danowska-Klonowska, 2004).**

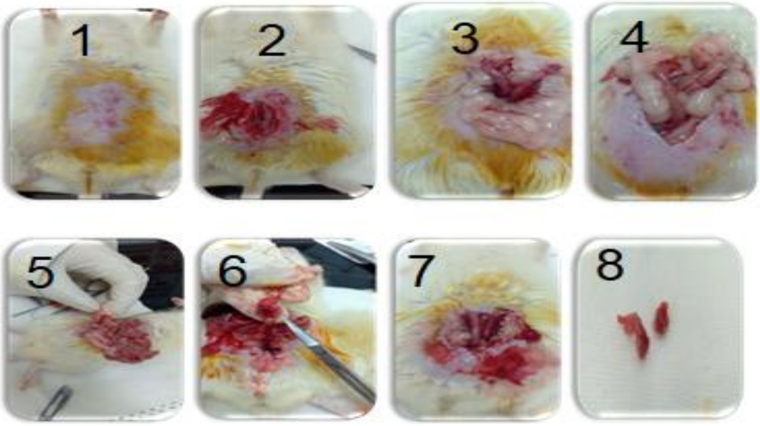


Photo (1): ovariectomy operation in rats.

Biological determination:

During the experimental period (8weeks), the amount of diet was recorded every day; In addition that weight was recorded weekly to determine food intake and body weight gain according to **(chapman et al., 1959).** Body weight gain was determinates using the following equation:

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

Organ:

Uterus were extracted from each rat, cleaned from adhesive matter and weighted according to method mentioned by **(Drury et al., 1967).**

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

Biochemical analysis:

At the end of the experiment period, the rats were fasted overnight then scarification of them by over dosage of halothane inhalation anesthetic agent then withdrawal of blood directly from heart by cardiac puncture and 2 ml of blood was added to anticoagulant tube and the rest in a blank tube for serum extraction left standing for 10 minutes to clot and centrifuged at 4000 rpm for 15 minutes to separate the serum which kept frozen at - 18°c till biochemical analyses.

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 **(Gindler and King, 1972)** and phosphorus **(Goodwin, 1970)** in serum. And urine samples were colorimetric ally determined using specific diagnostic reagent kits and measured on UV spectrophotometer. Serum ALP **(Nawawi and Girgis, 2002)** was estimated by colorimetric assay using specific enzyme kits).Serum osteocalcin concentration was measured by enzyme-linked immunosorbent assay according to **(Craciun et al., 2000).** Estrogen from blood samples was detected according to **(Setyawati et al., 2018).**

Bone analysis:

After sacrificing the rats Right femur was removed and the soft tissues were removed **(Doyle and Cashman, 2003).** And sample collected in formalin 10%for histopathological analysis and other samples were kept for **DEXA scan** in aluminum foil till scanning.

Histopathological examination:

At the end of the experimental period, the animals were sacrificed and the shafts of the femur were removed Sections were stained with hematoxylin and eosin and examined under a light microscope **(Bancroft and Gamble, 2008).**

Statistical analysis:

Data were represented as means ± SD. Statistical analysis was performed using computerized Statistical Package of Social Sciences (SPSS) program **(Snedecor and Cochran, 1989).**

Result and Discussion:

Data in Table (3) shows the chemical composition of SS and SP. The values for Water, protein, energy, total lipid, Ca, P, Fe, Mg and total saturated fatty acids.

SS and SP are good sources for the different food components including (protein; Total lipid(fat); Ash; Carbohydrate; Ca; Fe; Mg; P; Total saturated fatty acids; Total monounsaturated fatty acids; Isoflavones and Antioxidant.

Such data are in accordance with that obtained by **(Ji et al., 2019)** and **(Chinma et al., 2013)** which they reported that, SS and SP are a rich sources of protein, fatty acids, ca, p and active nutrients such as antioxidants and Isoflavones.

Table (3): Chemical composition value of whole, raw sesame seeds and Soy protein isolate:

Water (g)	Energy(kcal)	protein (g)	Total lipid (fat) (g)	Ash (g)	Carbohydrate (g)	Calcium, Ca (mg)	Iron, Fe (mg)	Magnesium, Mg (mg)	Phosphorus, P (mg)	Total saturated fatty acids (g)	Total monounsaturated fatty acids (g)	Isoflavones (mg)	Antioxidant (mg)
whole, raw sesame seeds													
4.59	574	16.7	48.6	4.35	21.4	985	14.55	354	659	6.15	17.7	-	413
Soy protein isolate													
4.78g	345	88.9	3.19	3.18	0	179	13.50	41	779	0.412	0.635	91.05	-

Biological determination:

Data on Table(4)show the Effect of Diets Fortified with SS and SP on Initial Weight (g); Final Weight (g); Feed intake (g/day/rat); BWG%; Uterus Weight (g) and OWG% of rats.

Feed intake (g/day/rat): Data in table (4) showed that the mean value of feed intake of healthy rat Control Negative about 18.5g/day/rat, while the mean value of feed intake in ovariectomized rats suffering from Osteoporosis decreased to 14.8g/day each rat in Control Positive. Treated rat with sesame seeds and soy protein increased the mean value of feed intake about 15.9g-17.2g for both treated groups if we compare that with Control Positive. Treating ovariectomized rats suffering from Osteoporosis rats with Sesame Seeds and Soy Protein and their combination led to slight increase in the mean value of feed intake, as compared to the Control Positive group.

Body weight gain%: The data in this table (4) revealed that, body weight gain% of the Control Positive group which ovariectomized and suffering from Osteoporosis increased significantly $p<0.05$, as compared to the control Negative group (healthy rat) (8.02%±1.92 for CN vs. 12.62%±2.08 for CP), respectively, feeding rats with Sesame Seeds and Soy Protein and their combination decreased the mean value of body weight gain% to 7.85%±1.12for 10%SS+50%SB and 6.73%±1.64 for 10%SS+100%SB than that of the control negative group.

Uterus Weight (g): Data in table (4) showed that, the mean value of Uterus Weight (g) of healthy rat Control Negative about 0.52g, while the mean value of Uterus Weight in ovariectomized rats suffering from Osteoporosis decreased to 0.14g in Control Positive rats. Treated rat with Sesame Seeds and Soy Protein slightly increased the mean value of Uterus Weight about 0.16g for both treated groups if we compare that with Control Positive.

OWG%: Sesame Seeds and Soy Protein and their combination affected on the mean value of Uterus Weight (g) are presented in table (4). We can observe that, BWG of ovariectomized groups hasn't significant statistically differences between them. The percent weight of Uterus was 0.06%, 0.07% and 0.08%.Respectively, comparing with non- ovariectomized rats which percentage is 0.25%.

According **to (Bigoniya *et al.*, 2012) and(Raeisi-Dehkordi *et al.*,2018)** showed that sesame seeds had a role in losing weight and organized blood lipids, Sesame ingestion has been shown to improve blood lipids in humans and anti-oxidative ability in animals. On the other hand **(Wei *et al.*, 2012)** confirmed that, despite a significant increase in food intake relative to OVX rats, the body weight of SOY fed rats was significantly reduced. Indicating that, through increased food consumption, soy has the ability to regulate body weight gain. On the other hand, the OVX / SOY fed group's body weight were equivalent (CN).

Geng *et al.*, (2019) reported that, Estrogen deficiency is a pivotal cause of postmenopausal bone loss and contributes to age-related bone loss beside obesity.

Beside that uterus weight recorded decrease in OVX group. And has slightly significant on increase on other treated groups according to these results were nearly similar with those obtained by **(Asarian and Geary, 2013) and (Chen *et al.*, 2015).**

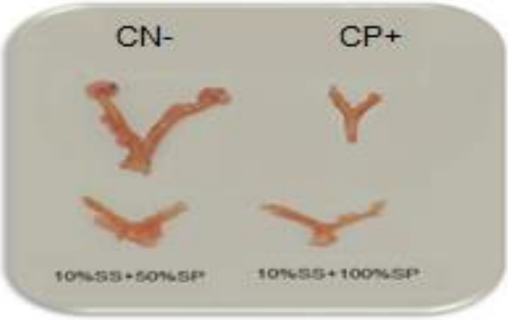


Photo (2): Effect of Diets Fortified with Sesame Seeds and Soy Protein on Uterus Weight (g) of rats suffering from Osteoporosis**.**

Table (4): Effect of Diets Fortified with SS and SP on Initial Weight (g), Final Weight (g), Feed intake (g/day/rat), BWG%, Uterus Weight (g) and OWG% of rats.

	Initial Weight (g)	Final Weight (g)	Feed intake (g/day/ rat)	BWG%	Uterus Weight (g)	OWG%
CN-	187	202	18.5	8.02%± 1.92[b]	0.52± 0.071[a]	0.25 %
CP+	198	223	14.8	12.62%± 2.08[a]	0.14± 0.029[b]	0.06 %
10%SS+50 %SB	191	206	15.9	7.85%± 1.12[b]	0.16± 0.038[b]	0.08 %
10%SS+10 0%SB	208	222	17.2	6.73%± 1.64[b]	0.16± 0.018[b]	0.07 %

(CN) Control Negative, **(CP)** Control Positive, **(SS)** Sesame seed**,** **(SP)** Soy protein
Values are expressed as mean **±SD.**

Biochemical analysis:

Data on Table (5) show the Effect of Diets Fortified with SS and SP on blood test (serum calcium (mg/dl); serum phosphorus (mg/dl); ALP (u/l); OC (µg/l) and estrogen) of rats suffering from Osteoporosis.

Serum calcium:

From table (5) it could be observed that, the mean values mean ±SD of serum calcium in the Control Positive group increased ($p<0.05$) as compared with Control Negative which is 11.83±0.33 vs 7.77±0.24.

in other wise Data presented showed that all treated OVX suffering from Osteoporosis with diet Fortified with Sesame Seeds and Soy Protein showed a significant decrease ($p<0.05$)in serum calcium, as compared to the Control Positive group.

Serum phosphorus:

Results in table (5) illustrated, the effect of Diets Fortified with SS and SP on serum phosphorus of rats suffering from Osteoporosis Rats which were fed on fortified diet led to significant slightly decrease ($p<0.05$)in serum phosphorus as compared to the Control Positive .

Increases in serum levels of Ca, P, ALP and OC induced by ovariectomy in rats, as reported in this study, were similar to the previously reported by (**Ramadan, 2014) and (Hassan *et al.*, 2013)** who concluded that increases in body weight gain and serum ALP and OC are due to estrogen deficiency in OVX rats and mice. Serum calcium, phosphorus, ALP and OC are commonly used as biochemical markers of bone formation (**Hinton *et al*., 2018).**

According to (**Wilson, 2016)** showed that the mean values of serum calcium and phosphorus increased on CP significantly $p<0.05$, as compared to healthy rats fed basal diet. On the other hand, serum calcium and phosphorus increased gradually with increasing the level of seeds.

Alkaline phosphatase (ALP):

Results in this table(5) revealed that, treating OVX rats suffering from Osteoporosis with diet Fortified with SS and SP 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 respect**, (Boulbaroud *et al.*, 2012)**revealed that ovariectomy significantly increased serum total The ALP activities increased significantly in OVX rats compared with the control Negative . A significant decrease in ALP activities was observed in both treated groups supplemented with flaxseed and SS at 10% ($p<0.05$).on otherwise (**Mukaiyama**

***et al.*, 2015).** Study presents an analysis demonstrates that phytoestrogens may offer the most potential for the prevention of bone loss by reducing loss of BMD also has significantly inhibit ALP and OC **(Pardhe *et al.*, 2017).**

Osteocalcin (OC):

Results in this table (5) revealed that, treating OVX rats suffering from Osteoporosis with diet Fortified with SS and SP showed a significant decrease ($p<0.05$) (OC), as compared to the Control Positive group which fed on basal diet only. It could be observed that, mean ±SD of (OC) in the CP group increased ($p<0.05$) as compared with CN. **Wanachewin *et al.*, (2017)** revealed that Sesamin down-regulates some osteoclastgenic related genes the expression of osteoclastgenic genes under sesamin treatment was examined and findings that identifies the inhibitory effects of sesamin on human osteoclast differentiation. The lignan effectively inhibits bone resorption .which declared in the decrease of OC level on serum at treated groups with sesame.On other hand **(Hinton *et al.*, 2018)** indicated that soy protein improved cortical bone biomechanical properties in female low-fit rats, regardless of ovarian hormone status. And there were a significant decrease on OC levels in treated rats which agree with our result.

Estrogen:

Results in this table (5) show that, treating OVX rats suffering from Osteoporosis with diet Fortified with SS and SP showed a significant increase ($p<0.05$) Estrogen, as compared to the Control Positive group which fed on basal diet only. Estrogen is the most potent inhibitor of osteoclastic bone resorption as **(Misra *et al.*, 2019)** show, so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis. The bilateral ovariectomy in rats caused dramatic decreases in the uterine weight, bone mineral content, density and biomechanical strength due to estrogen deficiency which cause osteoclast generation so increasing bone loss. (**Süntar and Küpeli Akkol, 2013)** and (**Lagari and Levis, 2014)** revealed that, Postmenopausal osteoporosis is commonly treated by ERT. (**Nayeem *et al.*, 2019)** findings suggest that micro nutrients such as isoflavones should be recognized as a new class of compounds that participate in calcium homeostasis in premenopausal women.

Table (5): Effect of Diets Fortified with SS and SP on blood test (serum Ca (mg/dl); serum P (mg/dl); ALP (u/l); OC (µg/l) and estrogen) of rats suffering from Osteoporosis.

	Serum calcium (mg/dl)	Serum phosphorus (mg/dl)	ALP (u/l)	OC (µg/l)	Estrogen
CN -	7.77± 0.24[c]	0.67± 0.49[b]	63.52± 1.996[c]	7.21± 0.01[d]	46.13± 1.01[a]
CP +	11.83± 0.33[a]	0.77± 0.24[a]	101.19± 1.42[a]	14.87± 0.03[a]	23.63± 1.92[d]
10%SS+50%SB	9.85± 0.51[b]	0.69± 0.29[b]	88.88± 2.973[b]	10.23± 0.02[b]	31.87± 2.05[c]
10%SS+100%SB	9.22± 0.11[b]	0.67± 0.11[b]	87.93± 2.245[b]	7.79± 0.03[c]	34.70± 0.88[b]

(CN) Control Negative, **(CP)** Control Positive, **(SS)** Sesame seed**, (SP)** Soy protein, **(ALP)** Alkaline Phosphatase, **(OC)** Osteocalcin.
Values are expressed as mean **±SD.**
Values which have different letters in each column differ significantly at **($p<0.05$)**

Urine Calcium:

Data on Table (6) show that, the mean values mean ±SD of Urine calcium in the CP group increased ($p<0.05$) as compared with CN.in otherwise all treated ovariectomized rats suffering from Osteoporosis with diet Fortified with SS and SP showed a significant decrease ($p<0.05$) in urine calcium as compared to the CP.

Urine phosphorus:

Illustrated outcomes in table (6) show, the effect of treating OVX rats led to significant decrease ($p<0.05$) in urine phosphorus as compared to the Control Positive.

The present analysis of **(Taku *et al.*, 2010)** revealed that soy isoflavone supplements significantly decreased the bone resorption marker urinary test. **(Park *et al.*, 2018)** declared that bone resorption and bone formation were the highest 5–10 years after menopause, and serum markers is more useful than urine marker among the bone resorption markers.

Ram and Jambu, (2019) aimed on his study that, find if urinary test can be used to diagnose osteoporosis. The result come to show that, Urinary test can very well be considered as a diagnostic test and can't be considered as a standard diagnostic test. It can be used as an adjuvant and as a screening test along with gold standard DEXA in diagnosing osteoporosis.

Table (6): Effect of Diets Fortified with SS and SP on Urine Ca and Urine P of rats suffering from Osteoporosis:

	urine calcium (mg/dl)	Urine phosphorus (mg/dl)
CN -	1.15±0.76[c]	54.42±0.92[c]
CP +	6.76±0.26[a]	60.01±0.26[a]
10%SS+50%SB	4.85±0.22[b]	58.92±0.88[b]
10%SS+100%SB	4.69±0.79[b]	58.69±0.42[b]

(CN) Control Negative, **(CP)** Control Positive, **(SS)** Sesame seed**, (SP)** Soy protein
Values are expressed as mean **±SD.**
Values which have different letters in each column differ significantly at **($p<0.05$)**

Histopathological examination:

The histopathological results of this study revealed that ovariectomy caused a decrease in thickness of the cortical compact bone in the middle shaft of the femur and of the trabeculae in cancellous bone in the head of the femur bone as we show in Photo (4) Histochemical results showed new bone formation in sections of rats treated with SS+SP. The best results were detected in sections of rats treated with a combination of the (10%SS+100%SP) as we show in Photo (6) Have a better ameliorating effect on ovariectomy-induced osteoporosis than (10%SS+50%SP) as shown in Photo (5)

In the present study, ovariectomy of rats induced a decrease in thickness of the shaft cortical bone and decrease in the number of osteocytes. Many osteoporotic cavities were also seen. The results of the present work are in agreement with those of (**Marcu *et al.*, 2011;(Pavel *et al.*2016) and (Abdelrazek *et al.*, 2019)** who reported that histomorphometric and statistical results of the outer cortical bone of OVX rats revealed significant decrease in the mean outer cortical bone thickness compared with CN- control rats. The femur bone of OVX rats also showed resorption cavities and irregularly surface containing osteoclasts and reduction in the cortical and trabecular bone thickness.

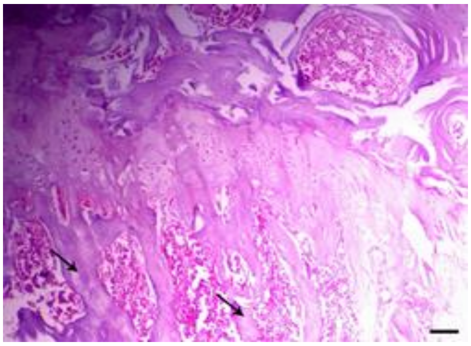
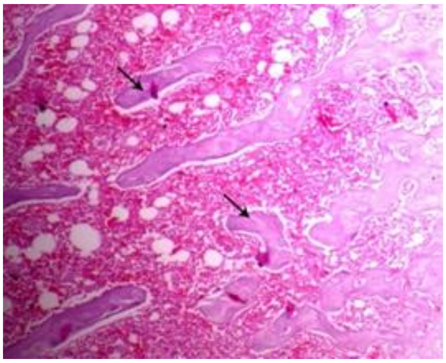


Photo (3): Bone section of control negative (CN) animal showing normal epiphyseal cartilage and cancellous or trabecular bone (arrows), H&E, X100 bar= 50 µm.



Photo(4): Bone section of control positive (CP) animal showing short, thin and separated spicules of trabecular bone (arrows), H&E, X100 bar= 50 µm.

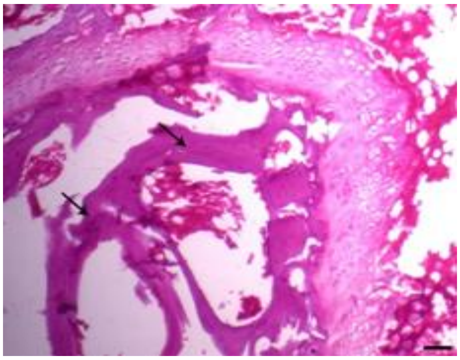


Photo (5): Bone section of G3 animal -treated with (10%SS+50%SP) showing thick and connected bone trabeculae (arrows), H&E, X100 bar= 50 µm.

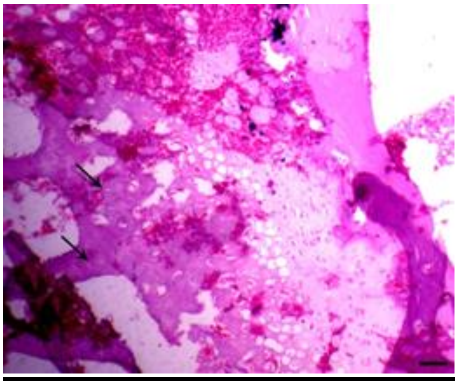


Photo (6): Bone section of G4 animal -treated with (10%SS+100%SP) showing normal cartilaginous layer and marked increase the thickness and density of trabecular bone (arrows), H&E, X100 bar= 50 µm.

DEXA Scan

Dual Energy X-ray Absorptiometry (DEXA) is considered as the benchmark method for specific diagnose of osteoporosis. Data on table (7) show the Effect of Diets Fortified with SS and SP on DEXA scanned and illustrated in photo(7) revealed that, treating OVX rats suffering from Osteoporosis with diet Fortified with Sesame Seeds and Soy Protein showed a significant increase on BMD, as compared to the Control Positive group which fed on basal diet only. As a result on table (7) it could be observed that, the values of (BMD) in CP group decreased as compared with CN. BMD was observed between the groups fed on our diet the best results in BMD was recorded for the group treated with (10%SS+100%SP) .we can observe that the best group result is nearly to the CN.

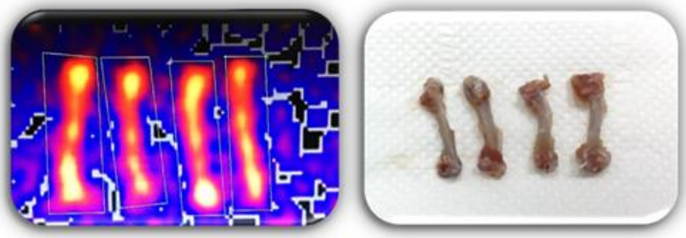


Photo (7): Bone result from NORLANDO DEXA scan model XR-46 report.

Our results were in accordance with that of (**Zhang *et al.*, 2017): (.Kim *et al.*, 2018): (Zaman *et al.*, 2018): (Bisaccia *et al.*, 2019). (Nazia Fathima *et al.*, 2020):And (Fathima *et al.*, 2020)** who found that DEXA scan is the best way for osteoporosis diagnose beside that phytoestrogens such as sesame seeds and soy protein might improve bone with ovariectomy-induced osteoporosis .The present study indicated that estrogen deficiency in rats subjected to bilateral ovariectomy caused bone metabolic alterations. The study also showed the ability of both SS and SP diets to have nearly the same positive and recovering effects on postmenopausal osteoporosis. So they could be considered as useful natural anti-osteoporotic agents in experimentally bilateral OVX-rats. Therefore it can be said that phytoestrogens, especially as food supplements, help to prevent osteoporosis in postmenopausal women around the world and who are involuntarily subjected to ovariectomy.

Table (7): Effect of Diets Fortified with SS and SP on DEXA scanned bone :

Region	BMD (g/cm)3	BMC (g)	Area (cm2)	Lean Mass (g)	Fat mass (g)
Control negative	0.1781	0.7638	3.932	0.0164	0.7409
Control positive	0.1557	0.5590	3.858	0.0191	0.9450
10%SS+50%SP	0.1601	0.6007	3.743	0.1307	0.8121
10%SS+100%SP	0.1690	0.6032	3.472	0.0726	0.7052

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

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