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Research Article

The Effect of Tahini and Sesame Perisperm Extract on the Rodent Post-Menopausal Osteoporosis Model

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ABSTRACT

Objective: This study aimed to examine the impact of sesame (tahini and sesame perisperm) on the postmenopausal bone mineral density ovariectomized rat model, as a potential agent for managing postmenopausal osteoporosis, either as a dietary supplement or as a substitute for hormone replacement therapy, without the severe side effects of other treatments.

Methods: Forty female, ten-month-old, Wistar rats were divided into control (n=10), Ovariectomized (OVX, n=10), Ovariectomized-Plus-Tahini (OVX+T, n=10) and Ovariectomized-Plus-Sesame Perisperm Extract (OVX+SPE, n=10), groups. Total and proximal tibial Bone Mineral Density (BMD) were measured by Dual-Energy X-ray Absorptiometry (DEXA) at baseline, 3 and 6 months afterward. Femurs were subjected to a Three-Point Bending Test (3PBT) at the end of the study, and the uterine weight and its ratio to body weight were examined.

Results: Both total and proximal tibial BMD values and percentage changes from the baseline of the OVX+T and OVX+SPE groups, were statistically significantly higher than those of the OVX group at all times. In 3PBT, the right femurs of the OVX+SPE group presented statistically significant increased value compared to the OVX group, for the indices Mises stress and bending stress, and the left femurs of the OVX+T group showed statistically decreased value for the torsional stress index.

Conclusion: Sesame tahini and perisperm extract improved postmenopausal tibial BMD loss. Ameliorative changes in biomechanical strength were also noted during the three-point bending test. Thus, sesame extract and tahini could consist important factors in the management of osteoporosis. Further research on the effects of sesame by-products in postmenopausal osteoporosis is imperative.

Keywords: Sesame perisperm; Tahini; Postmenopausal osteoporosis; Rat; Bone mineral density; DEXA; Biomechanical testing

INTRODUCTION

One in three postmenopausal women over the age of 50 globally suffer from osteoporosis, a multifactorial, clinically silent skeletal condition that causes bone fragility and an elevated risk of fractures [1-3]. Osteoporosis is a chronic, progressive disease, and the related fragility fractures place a heavy socioeconomic strain across the world [3-5]. Age and osteoporosis cause the balance of bone production and resorption to shift negatively [6]. Gradually, the minerals of the cortical layer and the bone cavity itself resorb, resulting in an initial loss of trabecular bone and a widening of the bone cavity. In post-menopausal women, estrogen insufficiency exacerbates bone resorption causing a further decline in bone strength [7-9]. Between menopause and age 75, women lose about 22% of their total bone mass. Of this loss, 13.3% is due to aging and 7.7% is due to estrogen deficiency [10].

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The general management of osteoporosis includes activities that increase balance and muscular strength, sufficient dietary intakes of essential bone-building elements such as calcium, vitamin D, protein and pharmacological interventions like Selective Estrogen-Receptor Modulators (SERMs, i.e., raloxifene), bisphosphonates (i.e. alendronate, ibandronate, risedronate, zoledronic acid), agents derived from parathyroid hormone (teriparatide) or intermittent administration of the hormone *per se*, human antibodies (denosumab), hormones like calcitonin or estrogens (Hormone Replacement Therapy (HRT) and vitamin D derivatives (i.e. alfacalcidol)). Nevertheless, using them comes with a known risk of a negative effect [11]. For example, raloxifene is linked to an increased incidence of deep vein thrombosis, and to a somewhat higher death rate following a stroke, oral bisphosphonates cause esophagitis, esophageal ulcers, jaw osteonecrosis, and abnormal femoral fractions, and finally, long-term menopausal HRT is associated with increased risk of coronary heart disease, breast cancer, stroke and dementia [11-13].

The severe side effects promote the research of non-pharmaceutical alternative solutions for bone preservation, osteoporosis prevention, and therapy. Some dietary components like unsaturated fatty acids, proteins, minerals, peptides, prebiotics, and phytoestrogens contribute to bone metabolism regulation and bone loss retention [14].

Phytoestrogens specifically, have structural and biological similarities to 17-estradiol, are able to bind to Estrogen Receptors (ER), with lesser though affinity than natural estrogens, and exert pro- or anti-estrogenic effects. An increasing body of evidence supported their usage in preventing and treating osteoporosis and menopausal symptoms. Since they are thought to be safe and effective, phytoestrogens are utilized both as dietary supplements and as a substitute for HRT, especially for individuals who are ineligible for HRT [15-17]. Clinical trials have not demonstrated an increase in the risk of breast cancer or an increase in endometrial hyperplasia after phytoestrogens usage. Lignans specifically, differ from HRT by appearing not to raise postmenopausal women's risk of clotting. Moreover, phytoestrogens can lessen the severity and the frequency of hot flushes. Additionally, some phytoestrogens enhance sleep and cognition, reduce vaginal atrophy, and enhance bone health. Thus, although pharmaco-vigilance is still necessary, phytoestrogens could provide a secure, less efficient though, substitute of HRT [18].

Sesame seeds and sesame oil are significant source of phytoestrogens and have phytoestrogenic properties similar to those of estradiol [19-21]. The main bioactive lignans found in sesame seeds are sesamin, sesamol, sesamolin, and sesaminol [22,23]. Pianjing et al., studied the estrogenic activities of sesamin, sesamolin and their metabolites (enterodiol, enterolactone, andsesamol) [24]. Sesamol could substantially increase progesterone receptor gene expression, whereas sesamin, sesamol and enterolactone, all considerably increased the expression of an estrogen-targeted gene, trefoil factor 1 (pS2) gene. Sesame oil has also been researched for intrascapular subcutaneous injections of 17-estradiol-3-benzoate every four days to increase plasma estradiol levels in the rat similarly in terms of concertation and duration, to the ovarian cycles [25-27].

There are many studies supporting the beneficial effects of the administration of sesame in osteoporosis [22,28-30]. Wu et al., concluded that sesame consumption benefits postmenopausal women by ameliorating free radical damage, blood lipid levels, and maybe even sex hormones levels [31]. Through TRAcP staining and hydroxyapatite resorption tests, sesame successfully suppressed osteoclast formation *in vitro*. Sesame decreased the protein expression of c-Fos and NFATc1, prevented the RANKL-induced activation of the NF-B and MAPK signaling pathways, and significantly inhibited the expression of genes that are expressed in osteoclasts [29]. In a study of Ma et al., sesamin enhanced osteoblastic differentiation of rat Bone Marrow Stromal Cells (BMSCs) by upregulating the Wnt/ß-catenin pathway, and bone structure [30]. Sesamin, isolated from *Sesamum indicum* seeds, has also been reported to protect the femoral head from osteonecrosis by inhibiting ROS-induced osteoblast apoptosis [32]. Inhibitory effect of sesamin on osteoclastogenesis in humans is supported as well [23,33]. Sesamin also demonstrated chondroprotective properties in a papain-induced osteoarthritis rat model [34]. Sesamin administration improved the expressions of Alkaline Phosphatase (ALP), Osterix (OSX), SRY-box 9 (SOX9), Runt-Related Transcription Factor 2 (RUNX2), Osteocalcin (OCN) and collage type I expression. Micro-CT confirmed improved Bone Mineral Density (BMD), Trabecular Number (Tb.N) and reduced Trabecular Spacing (Tb.Sp) in the femurs of OVX rats after intragastric administration of 80 mg/kg of sesamin [30]. In an another study of Hassan et al, feeding OVX rats on 10% sesame oil-supplemented diet for 2 months significantly improved serum, urine and bone Calcium (Ca), Phosphorus (P), Magnesium (Mg), serum estrogen, parathyroid hormone, osteocalcin, serum, urine and bone enzymes ALP and Acid Phosphatase (ACP), serum lipid profile (total lipids, total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol) and, as well as serum, urine and bone total protein, serum and urine urea and creatinine, bone mineral density and Nitric Oxide (NO) and urine hydroxyproline [28]. The sesame seed's ability to decrease plasma cholesterol is attributed to its high dietary fiber and linoleic acid content [31,35,36]. Finally, sesame oil has antioxidant properties due to its high levels of lignin, vitamin E (γ-tocopherol) and polyunsaturated fatty acids. Sesame oil has been shown to shield DNA from oxidative damage in *in vivo* setting [19,31,37,38]. Animals given defatted sesame flour or sesame oil presented less oxidative stress attributed to the synergistic interaction of lignans and tocopherol [31,39,40]. Other pharmacological effects linked to the lignans found in sesame seeds, like sesamin and sesamolin, include anti-inflammatory, antioxidant, anti-cancer, anti-hypertensive, anti-melanogenic, auditory protection, anti-cholesterol and protective properties to the heart, liver and kidneys [21,23,33,38]. Fat, protein, vitamins, minerals, and dietary fiber are all abundant in sesame seeds. Sesame seeds are known as the "crown of eight grains" and an "all-purpose nutrient bank" due to their high nutritional content [23,41].

This promising phytoestrogen, may be a safe, effective, dietary supplement or substitute for HRT. Thus, this study aimed to investigate the effect of sesame perisperm extract and tahini in the most commonly used in postmenopausal osteoporosis research, ovariectomized rat model [42]. The 10-month-old, skeletally mature rat, is considered proper animal model to that purpose [43-45].

MATERIALS AND METHODS

Laboratory animals

The General Directorate of Veterinary Services granted permission for the current experimental procedure (permit number. 5950/19-9-2014) in accordance with European Directive 2010/63/EU. The PREPARE and ARRIVE recommendations were taken into account [46,47].

Forty 3-month-old female Wistar rats with similar body weights were bought from the Hellenic Pasteur Institute. The animals were enclosed in transparent polycarbonate open-top cages (dimensions 45*30*20 cm), in groups of three or four, under standard laboratory settings (19-22°C, 55%-65% relative humidity, 15 air changes per hour, 12-hour light/dark cycle). Tap water and standard maintenance rodent pelleted diet were freely available. The rats were monitored daily throughout the duration of the study, with frequent veterinarian examinations, body weight and food intake measurements.

Study design

The rats were divided into four groups of ten each at the age of ten months: Control (n=10), Ovariectomized (OVX, n=10), Ovariectomized-Plus-Tahini (OVX+T, n=10) and Ovariectomized-Plus-Sesame Perisperm Extract (OVX+SPE, n=10). The total and proximal tibial Bone Mineral Density (BMD) of all animals was assessed by Dual-Energy X-ray Absorptiometry (DEXA), using a GE Lunar Prodigy Densitometer (General Electric Healthcare, Madison, WI, USA). Ten days following the initial DEXA measurement, all animals except the ones in control group, underwent ovariectomy. Two days after surgery, all animals started receiving a diet devoid of soy and soy byproducts. On the third and fourth postoperative days, tahini (sesame butter) and perisperm extract were progressively introduced to the pelleted diet of OVX+T and OVX+SPE groups respectively, to aid the animals become used to its flavor. The BMD measurements were conducted under general anesthesia and repeated after 3 and 6 months. Body weights were recorded every two weeks. The animals were euthanized following the third DEXA measurement by anesthesia overdose. All animals underwent post-mortem necropsy to examine the tissues for any potential aberrant pathological signs and to evaluate the effectiveness of OVX by uterine horn atrophy and the lack of ovarian remains. The uterus, abdominal fat, gastrocnemius muscle, heart, kidneys, brain and liver were separated from the surrounding tissues and weighed by blinded users. The femurs were also removed, wrapped with saline-soaked gauze and stored at -20^{0}C. The femurs were put through a three-point bending test and further biomechanical analysis.

Rat chow preparation and administration: To avoid any potential bias, such as the potent estrogenic effect of the diet on BMD, as mentioned previously, all groups were provided with chow that was soy- and soy byproduct-free (4RF21; Mucedola S.R.L., Milan, Italy). The oral administration was preferred since the chronic physical stress caused by other routes of administration is known to have an impact on sex hormones. Tahini was added to the rat diet of the OVX+T group at a dosage of 3 g/kg/day, taking into account that a rat eats around 20 g of food each day. The tahini dose was estimated using the formula of Human Equivalent Dose (HED, mg/kg)=animal dosage (mg/kg) *(animal Km/human Km). The human Km is 37 for a 60 kg human, the animal Km is 6 for rats, and 0.5 g/kg/day was utilized as the HED. Rat food was crushed into a mash-like consistency in a hammer mill using a 5 mm screen. A rotary mixer was used to combine this with tahini, and a Lister pellet press (Lister Co., Hardwicke, UK) was used to form the pellets. The sesame perisperm extract dose in the OVX+SPE was 200 mg/kg/day. Sesame perisperm was extracted with methanol (MeOH), employing an ultrasound-assisted extraction technique and the dried extract was similarly implemented to the pelleted diet. Every three days, the amount of tahini/sesame perisperm extract to be ingested was revised in accordance with the consumption per cage adjusted per rat.

Anesthesia: The rats were anesthetized by intramuscular injections of dexmedetomidine (Dexdomitor; Zoetis Hellas SA, Athens, Greece) and ketamine (Ketaset; Pfizer Hellas AE, Athens, Greece), at dosages of 0.25 mg/kg and 50 mg, respectively. Intramuscular administration of 1 mg/kg of atipamezole (Antisedan; Zoetis Hellas SA) was administrated following the ovariectomy and the first two DEXA measurements to the animals to reverse anesthesia.

Bone mineral density measurements (DEXA): The equipment was calibrated before measurements were taken for each group [44]. BMD is calculated by dividing the estimated bone mineral content in a Region of Interest (ROI) by the ROI's area (cm^2) using DEXA software [48,49]. The same blinded operator selected each ROI. The software's ROI tool was used in the current study to identify two ROIs. The first ROI (ROI 1), which included both cortical and trabecular bone, consisted the whole tibia. The second ROI (ROI 2: 0.19*0.19 mm^2) represented the proximal tibial metaphysis, which is abundant in trabecular bone, and was located close to the tibial plateau (3 mm). The proximal tibia was the skeletal site selected to be studied because it presents BMD changes early [44,50,51]. The system had an *in vitro* accuracy (coefficient of variation) of 0.5%.

Ovariectomy: Following the initial DEXA measurement, bilateral ovariectomy was carried out by a midline ventral incision, using aseptic techniques. Single interrupted 4-0 sutures were used. The control group was sham-operated. Preoperatively, carprofen for analgesia (Rimadyl; Zoetis Hellas SA) and enrofloxacin for chemoprophylaxis (Baytril; Bayer, Leverkusen, Germany) were given subcutaneously to rats at dosages of 4 mg/kg and 10 mg/kg, respectively.

Biomechanical testing: The Three-Point Bending Test (3PBT) on femurs is an acceptable, important test that measures how bones bend, revealing thus information about the mechanical characteristics of tiny animal bones [52]. Before the test, pictures of each femur were taken. A custom-created configuration to support the bones and MTS Insight loading frame were used for the 3PBT. A weight was applied steadily up at a speed of 1 mm/min to the bone's fracture. The supports were spaced 16 millimeters apart. One of the two bone pieces was properly processed and seen *via* a Leica MZ6 stereoscope in order to get the precise geometry of the shattered cross section. The biomechanical analysis is thoroughly prescribed by Pasiou et al., [53].

Statistical analysis

Data were expressed as mean ± Standard Deviation (SD). Shapiro-Wilks test examined the parameters' normal distribution. The two-way mixed ANOVA model was used using as factors 'the intervention' (between-group) and 'time' (within the group) for the analysis of BMD measurements using the Bonferroni correction for all pairwise comparisons either between or within groups. The comparison of the three-point bending results for each femur, the body weight, the organs weight, and the ratio of organ weight/body weight between groups were performed using the one-way ANOVA model. Pairwise comparisons were performed using the Bonferroni test. Sensitivity analysis of BMD measurements, concerning baseline balance between groups, was performed using 2 methods: a) the mean percentage change from baseline after 3 and 6 months respectively where comparison of percentage change from baseline of BMD parameters during the observation period between compared groups was analyzed using the one-way ANOVA model, pairwise comparisons were performed using the Bonferroni test. Kruskal Wallis and Mann-Whitney tests were used in case of violation of normality and b) the absolute change from baseline after 3 and 6 months respectively using analysis of covariance model ANCOVA using the absolute change from baseline in the measures of interest (3rd and 6th month) as the dependent variable, the groups as a factor and the baseline value of the measures as covariate. All tests are two-sided, and statistical significance was set at $p<0.05$. All analyses were carried out using the statistical package SPSS VR 21.00 (IBM Corporation, Somers, NY, USA).

RESULTS

DEXA measurements

Total tibia bone mineral density differences are presented in Table 1. Within all ovariectomized groups, the baseline total tibia BMD is gradual decreasing. The total tibia BMD of OVX+T group is significantly higher in baseline compared to both 3 and 6 months, and the third month total tibia BMD value is significantly higher to sixth month value. The total tibia BMD of OVX+SPE group is significantly higher in baseline compared to its value in 6 months, and the third month BMD value is significantly higher to sixth month value. Among groups, the BMD values of the control, OVX+T and OVX+SPE groups, are statistically significantly higher than those of the OVX group at all times. Similarly, the total tibia BMD percentage change from baseline of the control, OVX+T and OVX+SPE groups, are statistically significantly higher than this of the OVX group at all times. The only total tibia BMD percentage change from baseline among the OVX, OVX+T and OVX+SPE groups, that is not statistically significantly decreased compared to the control group, is the total tibia BMD percentage change of the OVX+SPE group from baseline to 3 months.

Proximal tibia bone mineral density differences are presented in Table 2. Within ovariectomized groups, the baseline proximal tibia BMD is significantly higher at all times and the proximal tibia BMD in 3 months are significantly higher to 6 months. Among groups, the proximal tibia BMD of the ovariectomized groups were statistically significantly decreased compared to the control group at all times. The proximal tibia BMD of the OVX+T and OVX+SPE groups were significantly increased compared to the OVX group at all times. Similarly, the proximal tibia BMD percentage change from baseline of the ovariectomized groups were statistically significantly decreased compared to the control group at all times. The proximal tibia BMD percentage change from baseline of the OVX+T and OVX+SPE groups were significantly increased compared to the OVX group at all times.

The absolute differences from baseline to 3 and 6 months in total and proximal tibial BMD between groups are presented in Table 3. At all times, the absolute BMD differences of the ovariectomized groups from baseline were significantly decreased compared to the control group. Likewise, the absolute BMD differences from baseline of the sesame-supplemented groups were significantly higher compared to the OVX groups throughout the study period. There was no statistically significant difference between the BMD values of the OVX+SPE and OVX+T groups at all times.

Three-point-bending

The results of the three-point-bending are presented in Table 4.

Table 1: Comparison of total tibia bone mineral density (g/cm^2) changes between groups during the observation period of 6 months. All values are presented as the mean ± standard deviation.

Group	Baseline	3 months	6 months	%change baseline-3	%change baseline-6
Control	0.243 ± 0.012	0.254 ± 0.009[b]	0.255 ± 0.013[b]	4.41 ± 4.15[b]	5.01 ± 8.03[b]
OVX	0.248 ± 0.010	0.217 ± 0.015[a,c]	0.205 ± 0.007[a,c,d]	-12.71 ± 5.08[a]	-17.46 ± 3.85[a]
OVX+T	0.246 ± 0.014	0.234 ± 0.018[a,b,c]	0.224 ± 0,024a,[b,c,d]	-4.25 ± 8.92[a,b]	-8.64 ± 8.91[a,b]
OVX+SPE	0.242 ± 0.009	0.237 ± 0.008[a,b]	0.226 ± 0.012[a,b,c,d]	-2.22 ± 2.83[b]	-6.52 ± 3.52[a,b]

Note: a: $p<0.05$ *vs.* control; b: $p<0.05$ *vs.* OVX; c: $p<0.05$ *vs.* baseline; d: $p<0.05$ *vs.* 3 months. BMD: Bone Mineral Density; OVX: Ovariectomy; OVX+T: Ovariectomized-Plus-Tahini; OVX+SPE: Ovariectomized-Plus Sesame Perisperm Extract.

Table 2: Comparison of proximal tibia bone mineral density (g/cm^2) changes between groups during the observation period of 6 months. All values are presented as the mean ± standard deviation.

Group	Baseline	3 months	6 months	%change baseline-3	%change baseline-6
Control	0.413 ± 0.022	0.426 ± 0.017	0.425 ± 0.027	3.28 ± 3.33	2.89 ± 5.56
OVX	0.405 ± 0.024	0.290 ± 0.026[a,c]	0.256 ± 0.021[a,c,d]	-28.21 ± 8.43[a]	-36.73 ± 5.95[a]
OVX+T	0.416 ± 0.012	0.367 ± 0.055[a,b,c]	0.347 ± 0.023[a,b,c,d]	-9.82 ± 7.45[a,b]	-16.46 ± 5.91[a,b]
OVX+SPE	0.406 ± 0.023	0.372 ± 0.035[a,b,c]	0.337 ± 0.028[a,b,c,d]	-8.38 ± 6.55[a,b]	-17.04 ± 3.77[a,b]

Note: a: p<0.05 *vs.* control; b: p<0.05 *vs.* OVX; c: p<0.05 *vs.* baseline; d: p<0.05 *vs.* 3 months. BMD: Bone Mineral Density; OVX: Ovariectomy; OVX+T: Ovariectomized-Plus-Tahini; OVX+SPE: Ovariectomized-Plus-Sesame Perisperm Extract.

Table 3: Comparison of absolute differences from baseline to 3 and 6 months in total and proximal tibial bone mineral density (g/cm^2) between groups. All values are presented as the mean ± standard deviation.

Variable	Group	Abs diff 3months-baseline[#]	p-value	Abs diff 6months-baseline[#]	p-value
		Mean (95%CI)		Mean (95%CI)	
Proximal	Control	0.015 (-0.002/0.032)	p<0.005	0.013 (-0.002/0.027)	p<0.005
	OVX	-0.119 (-0.136/-0.101)[a]		-0.151 (-0.167/-0.136)[a]	
	OVX+T	-0.038 (-0.054/-0.021)[a,b]		-0.066 (-0.081/-0.052)[a,b]	
	OVX+SPE	-0.037 (-0.054/-0.020)[a,b]		-0.071 (-0.085/-0.056)[a,b]	
Total	Control	0.009 (0.002/0.017)	p<0.005	0.011 (0.002/0.020)	p<0.005
	OVX	-0.030 (-0.037/-0.022)[a]		-0.042 (-0.051/-0.032)[a]	
	OVX+T	-0.011 (-0.018/-0.004)[a,b]		-0.021 (-0.030/-0.012)[a,b]	
	OVX+SPE	-0.007 (-0.014/0.000)[a,b]		-0.017 (-0.026/-0.008)[a,b]	

Note: a: p<0.05 *vs.* control; b: p<0.05 *vs.* OVX; [#]: Adjusted for baseline. OVX: Ovariectomy; OVX+T: Ovariectomized-Plus-Tahini; OVX+SPE: Ovariectomized-Plus-Sesame Perisperm Extract.

Table 4: Results of the three-point-bending test. All values are presented as the mean ± standard deviation.

Group	Control	OVX	OVX+T	OVX+SPE	p-value
Index			**Right femur**		
Mises stress (MPa)	191.92 ± 35.03	179.90 ± 34.75	200.86 ± 21.19	222.69 ± 26.19[a]	0.033
Bending stress (MPa)	191.81 ± 34.97	179.68 ± 34.78	200.60 ± 21.39	222.49 ± 26.23[a]	0.034
Torsional stress (MPa)	3.13 ± 2.37	3.95 ± 3.26	4.74 ± 3.36	4.34 ± 3.39	0.706
Cross-sectional area (mm)	5.02 ± 0.45	5.07 ± 0.23	4.82 ± 0.39	4.76 ± 0.40	0.244
Thickness (mm)	0.67 ± 0.09[a]	0.58 ± 0.05	0.63 ± 0.06	0.61 ± 0.08	0.046
			Left Femur		
Mises stress (MPa)	223.67 ± 44.90	189.67 ± 43.02	206.23 ± 26.74	211.14 ± 24.3	0.255
Bending stress (MPa)	223.50 ± 44.93	189.29 ± 43.00	206.17 ± 26.70	210.83 ± 24.28	0.25
Torsional stress (MPa)	4.26 ± 2.75	5.75 ± 4.19	2.03 ± 2.18[a,b]	5.82 ± 2.23	0.033
Cross-sectional area (mm)	4.81 ± 0.40	5.17 ± 0.36	4.74 ± 0.35	5.01 ± 0.47	0.103
Thickness (mm)	0.63 ± 0.09	0.59 ± 0.07	0.61 ± 0.06	0.63 ± 0.07	0.596

Note: a: p<0.05 *vs.* OVX; b: p<0.005 *vs.* OVX+SPE. OVX: Ovariectomy; OVX+T: Ovariectomized-Plus-Tahini; OVX+SPE: Ovariectomized-Plus-Sesame Perisperm Extract.

Right leg: There were no statistically significant differences among groups for the indices torsional stress, cross-sectional area, while there was for the thickness index; the control group had statistically significant increased value compared to OVX group. The OVX+SPE group presented statistically significant increased value compared to OVX group for the indices Mises stress and bending stress.

Left leg: There were no statistically significant differences among groups for the indices Mises stress, bending stress, cross-sectional area and thickness. For the Torsional stress index, the OVX+T group showed statistically significant decreased value compared to OVX+SPE and OVX groups.

Body, organ and relative weights

Body weight: The mean body weight changes are shown in Figure 1.

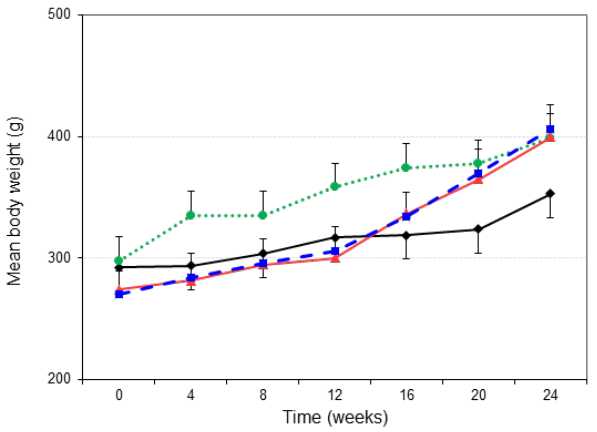

Figure 1: Mean body weight changes (g) throughout the study period. **Note:** (—●—): Control; (···●···): OVX; (—▲—): OVX+T; (– –■– –): OVX+SPE.

Until the 12th week, the OVX group weighted significantly higher compared to OVX+SPE and OVX+T groups. The following two months, the OVX groups weighted only significantly more than the control group. At 24 weeks there was no statistically significant difference among the body weights of the animals.

Organ weight: The weights of the gastrocnemius muscle and of the heart did not differ statistically significantly among the groups. The uterus, fat, brain and kidney weighted significantly more in the OVX group compared to the others.

Relative (ration organ/body) weight: The relative organ weight, which is the ratio of the organ weight to the animal's ultimate body weight, was also determined to ascertain whether sesame had a direct affect to an organ. The gastrocnemius relative weight was significantly higher in the control group compared to OVX and OVX+SPE groups. The relative weights of the brain and the heart were significantly higher in the control group compared to OVX+SPE and OVX+T groups. The relative weights of the uterus, liver and kidney were significantly higher in the control group compared to other groups. The fat relative weight was significantly decreased in the control group compared to the other groups.

DISCUSSION

As seen in PubMed, there is a constant interest the last 25 years in the research of phytoestrogens and postmenopausal osteoporosis. The phytoestrogens are researched as an alternative, natural hormone replacement treatment, free of the adverse effects of administrating estrogens or other pharmaceutical therapies.

Sesame seeds contain relatively high levels of lignans (mostly pinoresinol and lariciresinol), phytoestrogens that could lessen postmenopausal symptoms without increasing the risk of clotting [18,31,54-56]. In our study, the constant significantly ameliorated BMD values of sesame-supplemented groups compared to the OVX group in DEXA clearly confirm its beneficial effect. All tibial BMD values (BMD changes, percentage change from baseline, absolute differences from baseline) of the OVX+T and OVX+SPE groups, are statistically significantly higher than those of the OVX group at all times.

In 3PBT, there were indications of improved bone strength in the OVX+SPE and OVX+T groups. The right femurs of the OVX+SPE group presented statistically significant increased value compared to the OVX group, for the indices Mises stress and bending stress and the left femurs of the OVX+T group showed statistically decreased value for the torsional stress index.
These results, become more important taking into consideration the duration of the study, previous studies conducted in our laboratory, and that cortical bone changes occur more slowly to those of trabecular bone, demonstrating their effect on the bone strength at least nine months post ovariectomy [44,57-59]. The 3PBT and biomechanical analysis results are in accordance with other studies in which sesamol enhanced the mechanical stress parameters and bone ash content, and sesame seeds the femur biomechanical strength, respectively [56,60].

The fact that the OVX+T and OVX+SPE groups maintained significantly lowered bodyweight compared to OVX group up to 12 weeks may consist another positive indication, since body weight differences have been associated to bone strength in perimenopausal women [60,61]. Except from fat mass gain and bone loss in the OVX group, loss of muscular mass would be anticipated as well. This wasn't the case in our study. Regarding muscle mass, the weight of the gastrocnemius muscle showed no significant difference among groups. This may be due the duration of the study; longer study periods may be needed for muscular loss to be noted. Nevertheless, the relative weight of the gastrocnemius muscle did not differ significantly between OVX+T and control groups, possibly indicating an ameliorative effect of tahini on the gastrocnemius muscle. Both fat and uterine weights, and their relative weights, were significantly different among the control and all other groups. The significantly lowered to control group uterine weight is expected post-ovariectomy and it suggests that sesame byproducts may have weak or no estrogenic action on the uterus. Likewise, the significantly increased fat weight and its ratio to final total body weight of OVX, OVX+T and OVX+SPE groups compared to the control group indicate negligible effect on fat.

The six-month research period may consist a limitation as seen in similar studies [57-59]. Especially in rats older than 9 months old, that respond slowly to ovariectomy and administered treatments.

Cortical bone reacts to ovariectomy less quickly, compared to trabecular bone [26,42,44,57,59,62-65].

CONCLUSION

Furthermore, the proximal tibia studied in DEXA, stops growing at 15 months and the femoral mid-shaft studied in 3PBT, demonstrates an early transient increase in bone strength at 3 months that decreases after 9 months. Hence, a longer study period could result in even more significant differences in the biomechanical analysis, which is recommended for similar future studies.

Overall, sesame administration significantly ameliorated tibial bone loss in ovariectomized rats indicating the necessity to further research its role in postmenopausal osteoporosis.

AUTHORS' CONTRIBUTIONS

The authors of this article made the following contributions: SZ, AAN, DM, SM, EDP, ASD, SKK, PL, IAD, EC, GS and contributed to project design and data analysis; AG to statistical analysis; SZ, AAN, SM, SKK, IAD, AG, EC, GS and to the final review, article presentation and critical review of the article for important intellectual content; and SZ, DG, EDP, ASD, SKK, AEP, PP, PL, IAD to the conduct of experiments and laboratory tests; and DM, and SM to diet preparation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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