Pharmacological Reports 69 (2017) 1113–1119

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Oral administration of kaempferol inhibits bone loss in rat model of ovariectomy-induced osteopenia

Beata Nowak[a,*], Agnieszka Matuszewska[a], Anna Nikodem[e], Jarosław Filipiak[e], Marcin Landwójtowicz[b], Ewa Sadanowicz[b], Diana Jędrzejuk[b], Marta Rzeszutko[f], Krzysztof Zduniak[f], Tomasz Piasecki[c], Przemysław Kowalski[d], Wojciech Dziewiszek[a], Anna Merwid-Ląd[a], Małgorzata Trocha[a], Tomasz Sozański[a], Joanna Kwiatkowska[a], Marek Bolanowski[b], Adam Szeląg[a]

[a] Department of Pharmacology, Wroclaw Medical University, Wrocław, Poland
[b] Department of Endocrinology, Diabetology and Isotopes Therapy, Wroclaw Medical University, Wrocław, Poland
[c] Department of Epizootiology and Clinic of Birds and Exotic Animals, The Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wrocław, Poland
[d] Division of Pathomorphology and Oncological Cytology, Wroclaw Medical University, Wrocław, Poland
[e] Division of Biomedical Engineering and Experimental Mechanics, Wroclaw University of Technology, Wrocław, Poland
[f] Department of Pathomorphology, Wroclaw Medical University, Wrocław, Poland

ARTICLE INFO

Article history:
Received 7 October 2016
Received in revised form 22 January 2017
Accepted 5 May 2017
Available online 13 May 2017

Keywords:
Kaempferol
Bone mineral density (BMD)
Rats
Bone histomorphometry
Bone turnover
Bone mechanical properties

ABSTRACT

Background: Postmenopausal osteoporosis and osteoporotic fractures constitute an increasing problem in developing countries. Kaempferol, isolated from seeds of *Cuscuta chinensis*, is an active flavonoid inhibiting *in vitro* osteoclast activity. The aim of the presented research was an assessment of kaempferol effect on estrogen-deficiency-induced bone structure disturbances in rats.
Methods: The study was performed on 24 Wistar female rats divided into 3 groups: SHAM – rats undergoing a "sham" surgery, OVX-C – control group of animals that underwent ovariectomy, OVX-K – rats undergoing ovariectomy and receiving kaempferol for 8 weeks (from day 56 to day 112).
Results: In the OVX-K group, contrary to the OVX-C one, there was no significant decrease in femoral bone mineral density (BMD). A significant increase in Young's modulus was observed in the OVX-K group compared to the OVX-C (15.33 ± 2.51 GPa *vs.* 11.14 ± 1.93 GPa, $p < 0.05$). A decreased bone turnover was detected in the OVX-K group. Tissue volume ratio (BV/TV) and trabecular bone perimeter were increased in the OVX-K group compared to the OVX-C one (0.241 ± 0.037 *vs.* 0.170 ± 0.022, $p < 0.05$ and 15.52 ± 2.78 mm *vs.* 9.67 ± 3.07 mm, $p < 0.05$, respectively).
Conclusion: Kaempferol has a beneficial influence on estrogen-deficiency-induced disturbances of bone structure in rats.

Introduction

Postmenopausal osteoporosis is a prevalent systemic bone disorder characterized by low bone mass and deterioration of bone microarchitecture. Changes observed in bone structure and in its mineral content lead to increased bone fragility and a risk of fractures [1]. Estrogen deficiency is one of the most prevalent risk factors of osteoporosis in developed countries [2]. The number of patients suffering from osteoporosis and its complications will continue to grow with increasing population of elderly in industrialized countries. Osteoporotic fractures decrease patients' quality of life, and are responsible for premature disability and increased mortality rate [3]. Considering the fact that osteoporosis and its complications are associated with high socioeconomic costs, it is important to investigate new options of therapy and prophylaxis of the disease [4].

Estrogen deficiency plays an important role in development of osteoporosis and for that reason hormonal replacement therapy (HRT) has been proposed for treatment and prophylaxis of postmenopausal osteoporosis [5]. Despite the beneficial influence of HRT on bone metabolism its use is limited, because HRT

* Corresponding author.
E-mail address: beata.nowak@umed.wroc.pl (B. Nowak).

http://dx.doi.org/10.1016/j.pharep.2017.05.002
1734-1140/

increases the risk of breast and endometrium cancers. Bisphosphonates are strong and effective antiresorptive drugs, registered – among others – for osteoprososis management, but their use can be associated with osteonecrosis, bone microdamage and severe kidney and gastrointestinal side effects [6,7]. Drugs already registered for treatment of osteoporosis are effective, but long-term therapy with them may be associated with various side effects [8–10] that may lead to their necessary discontinuation and may affect patients compliance. Because of side effects of currently available antiosteoporotic drugs, many researchers focus on herbal drugs that may be useful in osteoporosis management. Some plant-derived substances, called "phytoestrogens" show estrogenic activity and may be promising drugs attenuating menopause-related signs and symptoms.

Kaempferol is a natural flavonoid isolated from *Cuscuta chinensis,* herb used in the Traditional Chinese Medicine among others to ameliorate symptoms of menopause and in therapy of postmenopausal osteoporosis in Chinese women [11,12]. Kaempefrol has been also identified, along with quercetin, in the extract of *Gingo biloba* leaves, that was demonstrated to increase bone mineral density in ovariectomized rats [13]. *In vitro* studies suggest that kaempferol is an estrogen-related receptor α and γ inverse agonist [14], and may reduce the risk of development of estrogen-dependent cancers more efficiently compared to pure hormonal replacement therapy (HRT). Wattel et al. suggested that kaempferol inhibits osteoclastic bone resorption *in vitro* [15]. Combination of both, above mentioned features makes kaempferol an ideal "candidate flavonoid" for osteoporosis management.

The purpose of the study was to investigate the influence of kaempferol on ovariectomy-induced bone disturbances in rats. We planned to assess the compound's effect on bone turnover on one hand, and on the other hand its influence on bone structure and bone mechanical properties, as only balance of bone turnover, bone structure and mineral content guarantees proper function of the skeletal tissue.

Material and methods

Animals

The study was performed on twenty four 12-week-old Wistar female rats. Animals were housed at a room temperature of 25 °C with 12:12-h light-day cycle. They were fed with a standard diet. Food and water were provided *ad libitum*. Acclimated rats were randomly assigned to one of three groups (eight animals each): surgically ovariectomized rats receiving either kaempferol (OVX-K) 5 mg/kg [16] in 0.9% saline solution 4 ml/kg daily or vehicle (OVX-C) 4 ml/kg *ig* daily, or the sham-operated group (SHAM) receiving vehicle 4 ml/kg *ig*. For the surgery, rats were anesthetized intraperitonally (*ip*) with xylazine (10 mg/kg) and ketamine (60 mg/kg). Then, a small skin incision was made and a complete bilateral ovariectomy was performed. Muscle and fascia were sutured separately using absorbable Safil 4.0, the skin was sutured using 4.0 silk. The sham group underwent the same surgical procedure but without ovariectomy. Eight weeks after surgery intragastrical (*ig*) administration of kaemferol and vehicle began and was continued for consecutive 56 days.

Body weights were checked once daily throughout the 16-week experimental period. On day 112 blood samples for serum isolation were collected. Serum was separated by centrifugation (at 1500 × *g*) and then stored at −70 °C until required for bone metabolic marker assays.

On day 112 animals were euthanized with pentobarbital (53.4 mg/kg *ip*). Femurs were obtained from each animal and femur index defined as ratio of femur weight and body weight ($\frac{femur\ \ mass[g]}{body\ \ mass[g]} \times 100\%$) was calculated. Right femurs were stored at −70 °C until required for dual-energy X-ray absorptiometry (DXA) and mechanical tests. Left femurs were fixed in buffered formaldehyde for further histological examination. The uterus was dissected from each animal and stored in buffered formalin for histological analysis.

The experiment was performed with the approval of Local Ethics Committee for Experiments on Animals.

Determination of bone mineral density (BMD)

Bone mineral density (BMD) of the right femur was measured by trained examiners by dual-energy X-ray absorptiometry (DXA) with Hologic DXA equipment (Hologic Discovery W 81507) using a software for small animals. Results were obtained as grams of mineral content per square centimeter of bone area (g/cm^2). The scanner was calibrated daily using a phantom provided by the manufacturer.

Measurement of bone relevant serum parameters

Serum total calcium and inorganic phosphate levels were measured in a certified laboratory using commercial tests performed according to their manufacturers' instructions.

Serum osteocalcin (OC) and beta C-terminated telopeptide of type I collagen (bCTX) levels (both sensitive biochemical markers of bone metabolism) were determined using commercial osteocalcin and CTX ELISA kits (Rat Osteocalcin ELISA Kit, USCN Life Science Inc. and Rat Beta-Crosslaps (bCTX) ELISA Kit, USCN Life Science Inc., respectively). Serum osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL) levels (both regulatory molecules involved in bone metabolism) were also determined using commercial ELISA kits (Rat Osteoprotegerin ELISA Kit, USCN Life Science Inc. and Rat Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL) ELISA Kit, USCN Life Science Inc., respectively). ELISA tests were performed according to their manufacturers' instructions.

Bone mechanical properties

Mechanical resistance of intact femurs were studied using a static four point bending test (Fig. 1) according to the protocol developed by us in the preliminary studies, described in detail in [17]. Mechanical properties of the femur were assessed using MTS

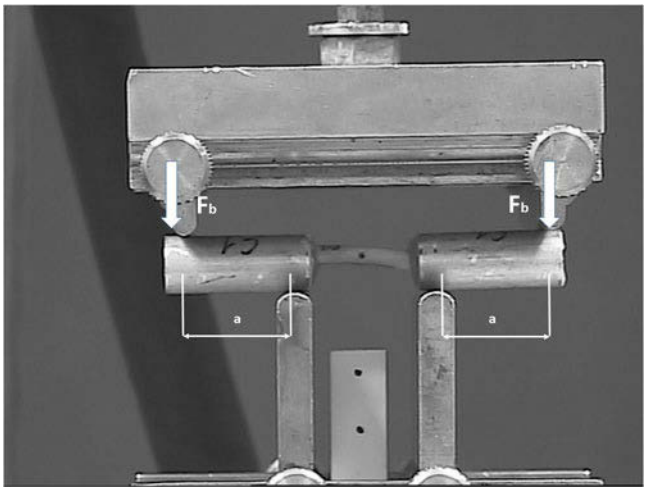

Fig. 1. Set-up for static four point bending test with examined femur in aluminum sleeves. Bending moment was calculated as: $M_b = F_b a$.

MiniBionix® 858 (MTS Systems – MN USA). The bones were mounted on the station using a special purpose instrumentation appropriate for the executed specific loading condition using the hydraulic operator MTS 242.02 with movement range of 100 mm. The bending moment Mb was applied perpendicularly to the long axis of the femur in the anatomical frontal plane. The load increased at the rate of 0.5 Nm/min until the breaking load was achieved. The braking load is defined as the load at which the bone actually broke. The reaction force was measured with the strain gauge MTS 661.19F-03 (nominal range 15 kN). The course of each measuring test and measuring data acquisition were controlled by means of the MTS FlexTest controller.

Deformation (strain) energy (U) and bending resistance (σ_{max}) were measured. Flexural yield stress (σ_b) was calculated using the formula: $\sigma_b = \frac{M_b}{W_b}[MPa]$ (Mb – bending moment [Nm], W_b – index of cross-section deformity in the response to bending [m^3]). W_b was determined for the transverse cross-section of medial part of femur. It was assumed the analyzed cross-sections were elliptic. To obtain the W_b value, transverse cross-sections of femurs bones were examined with microtomograph SkyScan 1172 100 kV, Bruker®.

Young's modulus (E) was calculated with the formula: $E = \frac{F \times a^2}{6 \times f_1 \times I} \times (3a + 2a)[MPa]$ (F – maximal force [N], I – moment of inertia [m^4], f_1–bending arrow [m], a, b – distance between holders [m], Fig. 1). Bone stiffness (k) was calculated with the formula: $k = E \times I$ (E – Young's modulus [MPa], I – moment of inertia [m^4]).

Histological preparations

Rat femurs were fixed in 10% neutral-buffered formaldehyde, then decalcified in 8% formic acid and 5.6% hydrochloric acid solution for 14 days. Next, the bones were embedded in paraffin and fixed, and 4-μm histological sections, stained with hematoxylin and eosin were subsequently prepared.

Rat uterus were fixed in 10% neutral-buffered formaldehyde. Next, they were embedded in paraffin and fixed, and 4-μm histological sections, stained with hematoxylin and eosin were subsequently prepared. Thickness of perimetrium, myometrium and endometrium was measured.

Bone histomorphometry

The histomorphometric examination of femurs was performed with respect to the 2012 update of the standardized nomenclature, symbols, and units for bone histomorphometry [18].

Histological slides were scanned with the NanoZoomer 2.0. In the NDP.view 2 software a representative tissue area (T.Ar) (of at least 1.7 mm^2) of femoral distal metaphysis trabecular bone, and the external cortical bone surface had been chosen for the analysis. That area was exported to a .tiff image format and trabecular bone area was thresholded in the ImageJ 1.50b software. Using the ImageJ, the total trabecular bone area (B.Ar) and the trabecular bone perimeter (B.Pm) were measured.

The bone volume to tissue volume ratio (BV/TV) was calculated as B.Ar/T.Ar, as it is numerically identical. Next we have calculated the bone surface to tissue volume ratio (BS/TV) as B.Pm/T.Ar*1.2 and the BS/BV ratio as BS/TV*BV/TV. The mean trabecular thickness (Tb.Th) was calculated as 2/BS/BV.

Statistical analysis

The significance of differences between values was estimated by the Student's *t*-test. P-value of less than 0.05 was considered to indicate statistically significant differences. Results are presented as the mean ± standard deviation (SD) unless otherwise stated.

All statistical analysis were performed using the STATISTICA software (data analysis software system), version 12, from StatSoft, Inc. (Krakow, Poland).

Results

In the beginning of the experiment, body weights were similar in analyzed groups. On day 56 there was a significant difference in body weight gains between ovariectomized (OVX-C and OVX-K) and sham-operated animals (61.34 ± 22.44% in ovariectomized animals *vs.* 33.56 ± 12.50% in SHAM group, $p < 0.05$) (Fig. 1). During the 8-week long period of administration of kaempferol normalization of weight gain in OVX-K group was observed (1.98 ± 2.38% in OVX-K *vs.* 3.08 ± 3.22% in SHAM, $p > 0.05$) whereas in OVX-C we observed persisting increase in weight gain (7.77 ± 3.14% in OVX-C *vs.* 3.08 ± 3.22% in SHAM, $p < 0.05$) (Fig. 2).

Bone parameters

Although femoral index was decreased in both ovariectomized groups (0.349 ± 0.021% in OVX-C and 0.348 ± 0.023% in OVX-K *vs.* 0.398 ± 0.033% in SHAM, $p < 0.05$), femoral BMD was significantly decreased only in the ovariectomized control group (0.233 ± 0.006 g/cm^2 *vs.* 0.246 ± 0.008 g/cm^2, $p < 0.05$) whereas no significant difference between the SHAM and the OVX-K group was detected (0.246 ± 0.008 g/cm^2 vs. 0.244 ± 0.009 g/cm^2, $p > 0.05$).

Bone mechanical properties

Results are presented in Fig. 3. Mean Young's modulus was 13.5% lower in the OVX-C group compared to the SHAM one and it was 19% higher in the OVX-K group than in the SHAM group, yet differences were not statistically significant. A significant difference in Young's modulus between OVX-C and OVX-K was detected (11.14 ± 1.93 GPa *vs.* 15.33 ± 2.51 GPa, $p < 0.05$).

Flexual yield stress was decreased in ovariectomized groups (30% in OVX-C and 20.5% in OVX-K) compared to the SHAM group, however the difference was not statistically significant.

The stiffness of femur bones was 25% higher in rats receiving kaempferol than in the SHAM group (0.145 ± 0.021 N/m^2 *vs.*

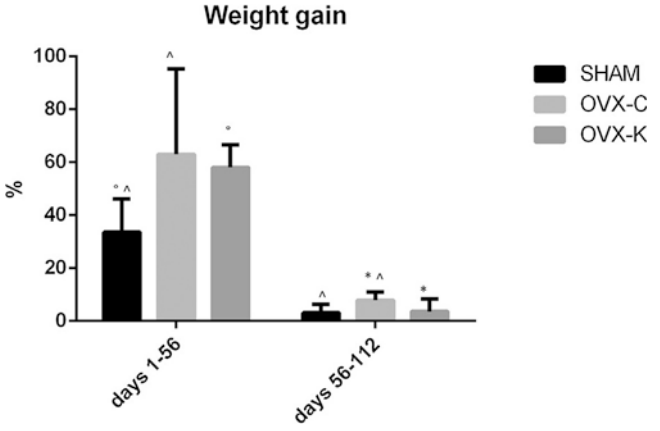

Fig. 2. Percentage weight gain in SHAM, OVX-C and OVX-K groups. SHAM – sham-operated group, OVX-C – ovariectomized control group, OVX-K – ovariectomized animals receiving kaempferol (5 mg/kg/day) from day 56 to 112. * – OVX-C *vs.* OVX-K, $p < 0.05$; ° – SHAM *vs.* OVX-K, $p < 0.05$; ^ – SHAM *vs.* OVX-C, $p < 0.05$.

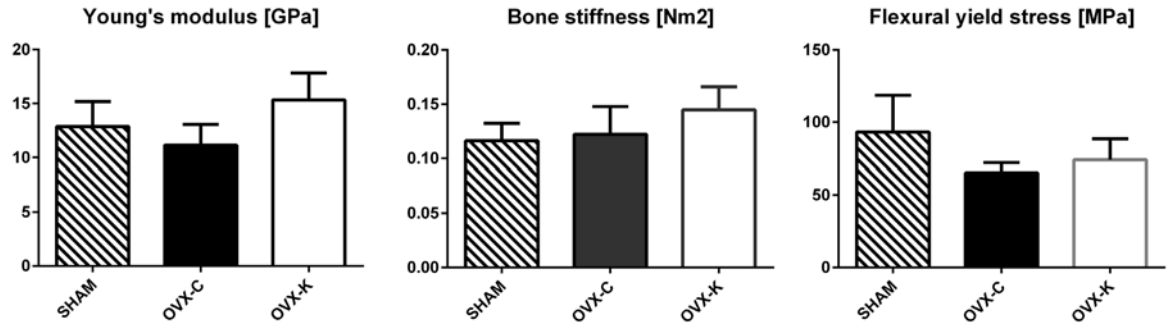

Fig. 3. Bone mechanical properties in SHAM, OVX-C and OVX-K groups. SHAM – sham-operated group, OVX-C – ovariectomized control group, OVX-K – ovariectomized animals receiving kaempferol (5 mg/kg/day) from day 56 to 112. $^{*}p < 0.05$.

0.116 ± 0.015 N/m^2, $p < 0.05$). No significant change of stiffness of femur bones was detected in the OVX-C group.

Bone serum parameters

Results are summarized in Table 1. There was no difference in serum total calcium and inorganic phosphate levels between analyzed groups. In the OVX-K group serum bCTX level was decreased by approx. 50% compared to the OVX-C group, however the difference was statistically insignificant. In the OVX-K group we detected a significant decrease in serum OC and RANKL levels compared to OVX-C and SHAM groups. Serum OC level in the OVX-C group was significantly lower than in the SHAM group. No difference between groups in serum OPG levels and OPG/RANKL ratio was found.

Histomorphometry

The results are summarized in Table 2.

In OVX-C group comparing to SHAM group we observed a decrease in BV/TV (−41%; $p = 0.016$), B.Ar. (−50%, $p > 0.05$), B.Pm. (−48%, $p = 0.011$) and BS/TV (−41%, $p = 0.006$). Trabecular thickness remained unchanged.

In kaempferol receiving group there was an increase in BV/TV (+41%, $p = 0.009$), B.Ar. (+91%, $p = 0.016$), B.Pm. (+60%, $p = 0.014$) and BS/TV (+19%, $p = 0.040$) comparing to OVX-C group.

Histological examination of uterus

Morphometric analysis of the thickness of perimetrium, myometrium and endometrium showed a significant uterine hypotrophy in ovariectomized animals (OVX-C: 1021 ± 128 μm, $p < 0.001$; OVX-K: 1271 ± 103 μm, $p < 0.001$) compared to sham-operated ones (2644 ± 245 μm). However, in the OVX-K group uterine hypotrophy was significantly less profound than in the OVX-C group (OVX-K *vs.* OVX-C, $p = 0.014$). Analyses of perimetrium, myometrium, and endometrium thickness revealed that differences between groups were mainly dependent on endometrium thickness.

Fig. 4 shows example photomicrography of a rat uterus from SHAM, OVX-K and OVX-C groups.

Discussion

Previous studies have shown that kaempferol, an active flavonoid isolated from seeds of *Cuscuta chinensis,* inhibits osteoclasts differentiation *in vitro* [15], and that its mechanism of action is at least partially a consequence of its inhibitory effect on the RANKL-mediated osteoclastogenesis [19,20]. RANKL-induced osteoclasts activation and differentiation of osteoclast precursor cells seem to be key factors in development of osteoporosis [21], and denosumab (anti-RANKL monoclonal antibody) has been already registered as antiosteoporotic drug [22]. In our study, kaempferol decreases RANKL level and this finding suggests that kaempferol may exert antiresorptive properties.

In vitro studies have shown that kaempferol may also exert an osteogenic effect [23], but our results did not support that hypothesis *in vivo* as OC level was decreased in OVX-K rats.

Ovariectomy-induced bone changes are characterized by increased bone turnover in the early phase, especially within first weeks after ovariectomy. In our study we did not reported increase in bone turnover markers (OC and bCTX) as we assessed their levels 16 weeks after ovariectomy, in the late phase of bone changes – probably stabilization of bone turnover has been already achieved. It would be of a great value if we monitored closely bone turnover markers during our 16 weeks observation. In our study, kaempferol, as a typical antiresorptive drug, inhibited bone turnover (decreased OC and bCTX levels) without affecting calcium and phosphorus concentrations. Similar effect on ovariectomy-

Table 1
The effect of long-term kaempferol administration on serum concentration of biochemical markers of bone metabolism.

Parameters	OVX-C	SHAM	OVX-K
Total calcium level [mg/dl]	9.58 ± 0.20	9.87 ± 0.06	9.63 ± 0.20
Inorganic phosphate level [mg/dl]	3.44 ± 1.10	3.47 ± 0.15	3.50 ± 0.69
bCTX [pg/ml]	119.3 ± 42.42	97.06 ± 74.65	58.87 ± 42.74
OC [pg/ml]$^{*,\#}$	255.21 ± 60.23	342.10 ± 94.02	164.98 ± 36.95
RANKL [pg/ml]*	234.82 ± 23.10	275.54 ± 38.78	195.89 ± 32.29
OPG [pg/ml]	362.0 ± 199.6	448.4 ± 158.6	352.2 ± 219.08
OPG/RANKL ratio	1.600 ± 0.845	1.769 ± 0.901	1.860 ± 1.010

OVX-C – ovariectomized control group, SHAM – sham-operated group, OVX-K – ovariectomized animals receiving kaempferol (5 mg/kg/day) from day 56 to 112, bCTX – beta C-terminated telopeptide of type I collagen, OC – osteocalcin, RANKL – Receptor Activator of Nuclear Factor Kappa B Ligand, OPG – osteoprotegerin. Results presented as: mean ± SD (standard deviation). *OVX-C *vs.* OVX-K, $p < 0.05$. $^{\#}$OVX-C *vs.* SHAM, $p < 0.05$.

Table 2
Histomorphometric parameters in groups OVX-C, SHAM and OVX-K.

Parameters	OVX-C	SHAM	OVX-K
Tb.Th. [μm]	40.99 ± 4.06	40.98 ± 8.03	48.50 ± 8.06
BV/TV[*,#]	0.17047 ± 0.022179	0.28931 ± 0.068502	0.24102 ± 0.036659
B.Ar. [mm^2][*]	0.23758 ± 0.080028	0.47472 ± 0.176704	0.45534 ± 0.124913
T.Ar. [mm^2]	1.42 ± 0.53	1.62 ± 0.36	1.87 ± 0.34
B.Pm. [mm][*,#]	9.67 ± 3.07	18.57 ± 3.86	15.52 ± 2.78
BS/TV[mm^2/mm^3][*,#]	8.36 ± 1.27	14.19 ± 2.45	9.98 ± 8.36
BS/BV [mm^2/mm^3]	49.15 ± 4.69	50.16 ± 9.25	42.18 ± 6.87

OVX-C – ovariectomized control group, SHAM – sham-operated group, OVX-K – ovariectomized animals receiving kaempferol (5 mg/kg/day) from day 56 to 112, B.Ar. – total trabecular bone area, B.Pm. – trabecular bone perimeter, BS – bone surface, BV – bone volume, BV/TV – tissue volume ratio, T.Ar. – representative tissue area, Tb.Th. – mean trabecular thickness, TV – tissue volume. Results presented as: mean ± SD (standard deviation). [*]OVX-C *vs.* OVX-K, $p < 0.05$. [#]OVX-C *vs.* SHAM, $p < 0.05$.

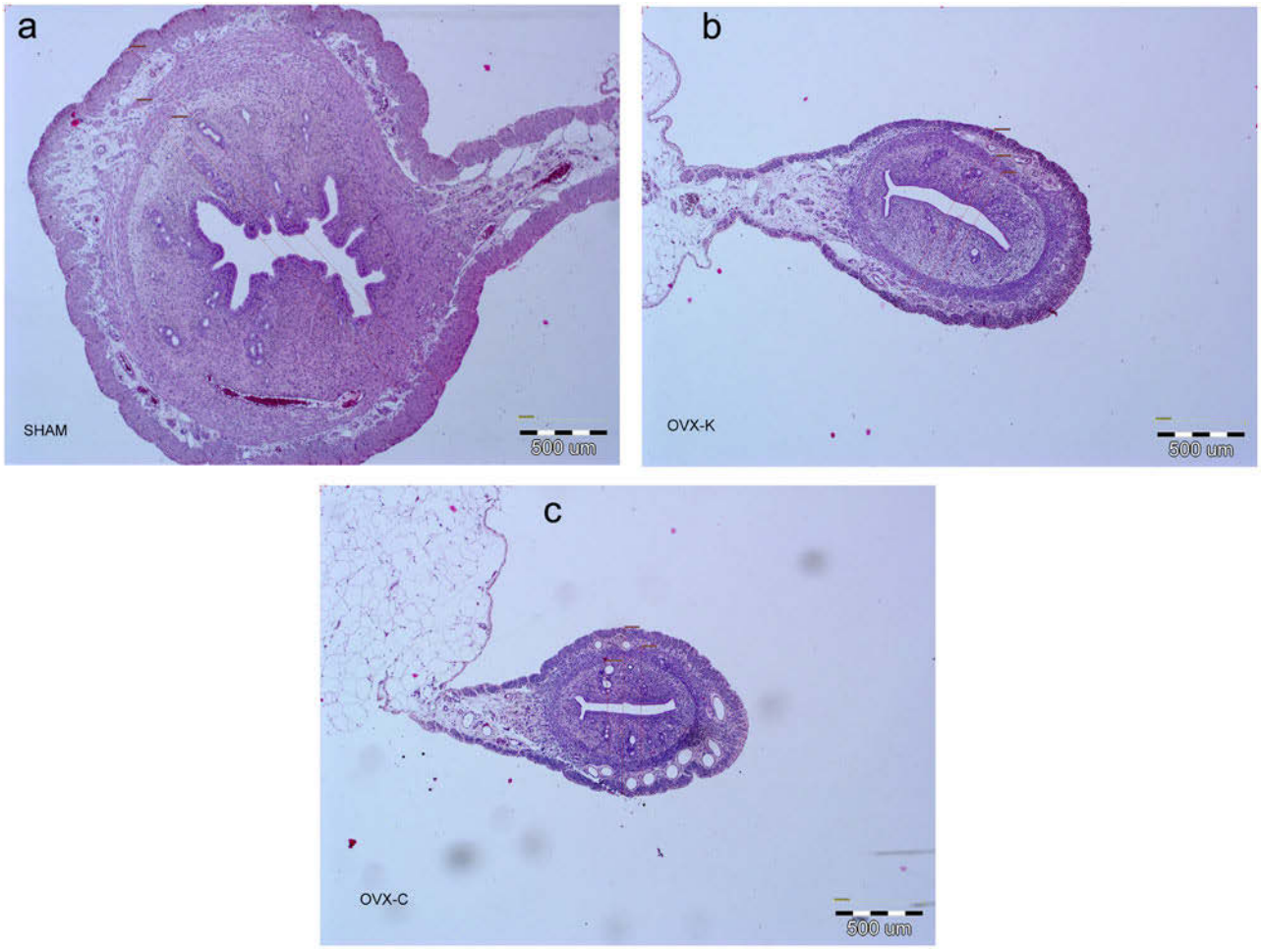

Fig. 4. Thickness of uterus layers in different experimental groups (HE).

induced bone changes is achieved with estrogen administration. The meta-analysis presented by Taku et al. demonstrated that soybeans izoflavones decrease the level of bone resorption markers [24]. Sathyapalan et al. reported that soybeans isoflavones, similarly to kaempferol in our study, reduced not only bone-resorption markers, but also bone-formation markers [25].

Kaempferol (5 mg/kg) prevented ovariectomy-induced bone loss in the femur and increased bone stiffness and femoral Young's modulus. Trivedi et al. reported an improvement in mechanical properties of vertebrae in kaempferol receiving rats [16], however they did not examine the influence of kaempferol on femoral bones. A beneficial influence of soybeans flavonoids, among others daidzein and genistein, on BMD and bone mechanical properties have been reported by other authors [26]. The meta-analysis concerning ovariectomy-induced osteoporosis in rats revealed that phytoestrogen treatment could prevent BMD decrease in femurs mainly through inhibiting bone resorption, however most of the analyzed papers reported research on izoflavones [27].

Histomorphometrical changes in OVX-C are similar to ovariectomy-induced changes reported by other authors [28]. In our study

kaempferol (5 mg/kg) improved various histomorphological parameters, *e.g.* B.Ar. (total trabecular bone area), B.Pm. (trabecular bone perimeter), BS/TV ratio (bone surface to tissue volume ratio) and BV/TV ratio (bone volume to tissue volume ratio). Increase in BV/TV ratio has been also reported in soybeans isoflavones treated animals with ovariectomy-induced osteoporosis [29,30].

In our experiment, a long administration of kaempferol (5 mg/kg) did not cause any endometrial hypertrophy. A similar observation in Spague-Dawley rats was reported by Trivedi et al. [16]. Daidzein, one of the most abundant isoflavons, similarly do kaempferol, does not cause any endometrial hypertrophy [26]. An absence of estrogenic influence of kaempferol on the uterus makes that plant derivate safer than other phytoestrogens, as it is not expected to promote development of endometrial and/or breast cancer. The observed effect on the uterus may be a consequence of the compound's progestogenic effect reported by Toh et al. [31]. Kaempferol is reported to be an estrogen-related receptor α and γ inverse agonist [14] and that may prevent it from inducing endometrial hypertrophy. Moreover, Kim et al. reported that kaempferol suppressed estrogen-induced breast cancer cell growth [32]. Contrary to kaempferol, genistein, the most potent soybean isoflavone, seems to have a trophic effect similar to that of estrogens on the uterus of sterilized rats [33].

We observed as well that kaempferol (5 mg/kg) reduced ovariectomy-induced weight gain. In recent studies genistein increased bone mass in obese osteoporotic ob/ob mice but it did not affect obesity itself [34]. However, Kim et al. reported that genistein reduced food intake and weight gain in ovariectomized mice [35].

The beneficial influence of kaempferol on ovariectomy-induced bone changes needs further research. It would be of a great value if we could compare kaempferol efficacy to other drugs used in osteoporosis prophylaxis and treatment, *e.g.* HRT and bisphosphonates. The limitation of the study is the fact that only one dose of kaempferol was investigated. We plan to compare various doses in the future.

Conclusion

Taken together, the results from this study demonstrate that kaempferol prevent ovariectomy-induced bone loss in rats probably due to its antiresorptive properties. Apart from that kaempferol has a beneficial influence on ovariectomy-induced body weight gain and does not cause endometrial hypertrophy.

Funding

This study was founded by Wroclaw Medical University (grant number ST808).

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Khajuria DK, Razdan R, Mahapatra DR. Drugs for the management of osteoporosis: a review. Rev Bras Reumatol 2011;51(4):365–82.
[2] Puszczewicz M. Rheumatology great internal medicine. Medical Tribune Polska; 2011 [in polish].
[3] van Staa TP, Geusens P, Bijlsma JWJ, Leufkens HGM, Cooper C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. Arthritis Rheum 2006;54(10):3104–12.
[4] Becker DJ, Kilgore ML, Morrisey MA. The societal burden of osteoporosis. Curr Rheumatol Rep 2010;12(3):186–91.
[5] Rossini M, Adami S, Bertoldo F, Diacinti D, Gatti D, Giannini S, et al. Guidelines for the diagnosis, prevention and management of osteoporosis. Reumatismo 2016;68(1):1–39.
[6] McClung M, Harris ST, Miller PD, Bauer DC, Davison KS, Dian L, et al. Bisphosphonate therapy for osteoporosis: benefits, risks, and drug holiday. Am J Med 2013;126(1):13–20.
[7] Bejhed RS, Kharazmi M, Hallberg P. Identification of risk factors for bisphosphonate-associated atypical femoral fractures and osteonecrosis of the jaw in a pharmacovigilance database. Ann Pharmacother 2016;50(8):616–24.
[8] Odvina CV, Zerwekh JE, Rao DS, Maalouf N, Gottschalk FA, Pak CYC. Severely suppressed bone turnover: a potential complication of alendronate therapy. J Clin Endocrinol Metab 2005;90(3):1294–301.
[9] Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. JAMA 2002;288(3):321–33.
[10] Lewiecki EM, Miller PD, Harris ST, Bauer DC, Davison KS, Dian L, et al. Understanding and communicating the benefits and risks of denosumab, raloxifene, and teriparatide for the treatment of osteoporosis. J Clin Densitom 2014;17(4):490–5.
[11] Donnapee S, Li J, Yang X, Ge A, Donkor PO, Gao X, et al. Cuscuta chinensis lam.: a systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional herbal medicine. J Ethnopharmacol 2014;157:292–308.
[12] Yang L, Chen Q, Wang F, Zhang G. Antiosteoporotic compounds from seeds of cuscuta chinensis. J Ethnopharmacol 2011;135(2):553–60.
[13] Trivedi R, Kumar A, Gupta V, Kumar S, Nagar GK, Romero JR, et al. Effects of egb 761 on bone mineral density, bone microstructure, and osteoblast function: possible roles of quercetin and kaempferol. Mol Cell Endocrinol 2009;302(1):86–91.
[14] Wang J, Fang F, Huang Z, Wang Y, Wong C. Kaempferol is an estrogen-related receptor alpha and gamma inverse agonist. FEBS Lett 2009;583(4):643–7.
[15] Wattel A, Kamel S, Mentaverri R, Lorget F, Prouillet C, Petit J, et al. Potent inhibitory effect of naturally occurring flavonoids quercetin and kaempferol on in vitro osteoclastic bone resorption. Biochem Pharmacol 2003;65(1):35–42.
[16] Trivedi R, Kumar S, Kumar A, Siddiqui JA, Swarnkar G, Gupta V, et al. Kaempferol has osteogenic effect in ovariectomized adult sprague-dawley rats. Mol Cell Endocrinol 2008;289(1–2):85–93.
[17] Nowak B, Matuszewska A, Filipiak J, Nikodem A, Merwid-Ląd A, Pieśniewska M, et al. The influence of bexarotene, a selective agonist of the retinoid receptor x (rxr), and tazarotene, a selective agonist of the retinoid acid receptor (rar), on bone metabolism in rats. Adv Med Sci 2016;61(1):85–9.
[18] Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the asbmr histomorphometry nomenclature committee. J Bone Miner Res 2013;28(1):2–17.
[19] Lee W, Lee E, Sung M, Yoo W. Kaempferol inhibits IL-1β-stimulated, rankl-mediated osteoclastogenesis via downregulation of mapks, c-fos, and nfatc1. Inflammation 2014;37(4):1221–30.
[20] Pang JL, Ricupero DA, Huang S, Fatma N, Singh DP, Romero JR, et al. Differential activity of kaempferol and quercetin in attenuating tumor necrosis factor receptor family signaling in bone cells. Biochem Pharmacol 2006;71(6):818–26.
[21] Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93(2):165–76.
[22] Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 2009;361(8):756–65.
[23] Prouillet C, Mazière J, Mazière C, Wattel A, Brazier M, Kamel S. Stimulatory effect of naturally occurring flavonols quercetin and kaempferol on alkaline phosphatase activity in mg-63 human osteoblasts through erk and estrogen receptor pathway. Biochem Pharmacol 2004;67(7):1307–13.
[24] Taku K, Melby MK, Kurzer MS, Mizuno S, Watanabe S, Ishimi Y. Effects of soy isoflavone supplements on bone turnover markers in menopausal women: systematic review and meta-analysis of randomized controlled trials. Bone 2010;47(2):413–23.
[25] Sathyapalan T, Aye M, Rigby AS, Fraser WD, Thatcher NJ, Kilpatrick ES, et al. Soy reduces bone turnover markers in women during early menopause–a randomized controlled trial. J Bone Miner Res 2016, doi:http://dx.doi.org/10.1002/jbmr.2927 [Epub ahead of print].
[26] Fonseca D, Ward WE. Daidzein together with high calcium preserve bone mass and biomechanical strength at multiple sites in ovariectomized mice. Bone 2004;35(2):489–97.
[27] Fu S, Zeng G, Zong S, Zhang Z, Zou B, Fang Y, et al. Systematic review and meta-analysis of the bone protective effect of phytoestrogens on osteoporosis in ovariectomized rats. Nutr Res 2014;34(6):467–77.
[28] Li Q, Hu B, Huang L, Chen Y, Weng L, Zheng H, et al. Effects of low doses of hydrochloride tetracycline on bone metabolism and uterus in ovariectomized rats. Acta Pharmacol Sin 2003;24(6):599–604.
[29] Dai R, Ma Y, Sheng Z, Jin Y, Zhang Y, Fang L, et al. Effects of genistein on vertebral trabecular bone microstructure, bone mineral density, microcracks, osteocyte density, and bone strength in ovariectomized rats. J Bone Miner Metab 2008;26(4):342–9.
[30] Hooshmand S, Juma S, Arjmandi BH. Combination of genistin and fructooligosaccharides prevents bone loss in ovarian hormone deficiency. J Med Food 2010;13(2):320–5.

[31] Toh MF, Mendonca E, Eddie SL, Endsley MP, Lantvit DD, Petukhov PA, et al. Kaempferol exhibits progestogenic effects in ovariectomized rats. J Steroids Horm Sci 2014;5(3):136.
[32] Kim S, Hwang K, Choi K. Treatment with kaempferol suppresses breast cancer cell growth caused by estrogen and triclosan in cellular and xenograft breast cancer models. J Nutr Biochem 2016;28:70–82.
[33] Francisco AMC, Carbonel AF, Simões RS, Soares JMJ, Baracat EC, Haidar MA. Do extracts of oral soybean augment the trophic effect of estrogen on the rat uterus? Climacteric 2013;16(1):161–8.
[34] Michelin RM, Al-Nakkash L, Broderick TL, Plochocki JH. Genistein treatment increases bone mass in obese, hyperglycemic mice. Diabetes Metab Syndr Obes 2016;9:63–70.
[35] Kim H, Nelson-Dooley C, Della-Fera MA, Yang J, Zhang W, Duan J, et al. Genistein decreases food intake, body weight, and fat pad weight and causes adipose tissue apoptosis in ovariectomized female mice. J Nutr 2006;136 (2):409–14.