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Royal jelly and bee pollen decrease bone loss due to osteoporosis in an oophorectomized rat model

Arı sütü ve arı poleni ooferektomi yapılan sıçan modelinde osteoporoza bağlı kemik kaybını azaltır

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Objectives: In this study, we aimed to investigate whether royal jelly and bee pollen reduce the bone loss due to osteoporosis in oophorectomized rat model.

Materials and methods: Thirty-two female Sprague-Dawley mature rats at six-month-old, weighing 180-260 g were used in the study. The rats were divided into four groups: Sham-operation group, only oophorectomy group, oophorectomy in combination with royal jelly group, and oophorectomy and bee pollen group. The rats were sacrified within 12 weeks following surgery. Bone mineral density (BMD) was measured and blood samples were collected for biochemical analysis before sacrification. Following sacrification, uterine weights were measured and tissue samples were taken to determine bone calcium and phosphate level with imaging through scanning electron microscope.

Results: The uterine weights of the rats were found higher in Sham-operation group than the other groups. The difference among the groups was statistically significant (p=0.001). Total body BMD results were similar in all groups and there was not statistically significant difference (p=0.19). The lumbar spine and proximal femur BMD results were statistically significantly higher in the royal jelly and bee pollen groups, compared to only oophorectomy group (p=0.001). Bone tissue calcium and phosphate levels were higher in royal jelly and bee pollen groups.

Conclusion: Royal jelly and bee pollen decrease the bone loss due to osteoporosis in oophorectomized rat model. These results may contribute to the clinical practice.

Key words: Animal experiments; osteoporosis; pollen; postmenopausal; royal jelly.

Amaç: Bu çalışmada arı sütü ve arı poleninin ooferektomi yapılan sıçan modelinde osteoporoza bağlı kemik kaybını azaltıp azaltmadığı araştırıldı.

Gereç ve yöntemler: Bu çalışmada altı aylık, ağırlıkları 180-260 gram arasında değişen 32 adet Sprague-Dawley cinsi dişi sıçan kullanıldı. Sıçanlar dört gruba ayrıldı: Sham-operasyon grubu, yalnızca ooferektomi yapılan grup, ooferektomi ile birlikte arı sütü uygulanan grup, ooferektomi ile birlikte arı poleni uygulanan grup. Sıçanlar cerrahi işlemden 12 hafta sonra sakrifiye edildi. Sakrifikasyondan önce kemik mineral yoğunluğu (KMY) ölçüldü ve biyokimyasal analiz için kan örnekleri alındı. Sakrifikasyondan sonra uterus ağırlıkları ölçüldü ve taramalı elektron mikroskobunda görüntüleme ile kemik dokuda kalsiyum ve fosfor düzeyini belirlemek için doku örnekleri alındı.

Bulgular: Sıçanların uterus ağırlıkları Sham-operasyon grubunda, diğer gruplara kıyasla, daha fazla bulundu. Gruplar arasındaki bu fark istatistiksel olarak anlamlı idi (p=0.001). Tüm vücut KMY sonuçları bütün gruplarda birbirine benzerdi ve istatistiksel olarak anlamlı bir fark saptanmadı (p=0.19). Lomber vertebra ve proksimal femur KMY sonuçları, arı sütü ve arı poleni uygulanan gruplarda, yalnızca ooferektomi yapılan gruba kıyasla, istatistiksel olarak anlamlı derecede yüksekti (p=0.001). Kemik dokudaki kalsiyum ve fosfor düzeyleri, arı sütü ve arı poleni uygulanan gruplarda daha yüksek olarak saptandı.

Sonuç: Arı sütü ve arı poleni, ooferektomi yapılan sıçan modelinde osteoporoza bağlı kemik kaybını azaltır. Elde edilen bulguların klinik uygulamaya katkısı olabilir.

Anahtar sözcükler: Hayvan deneyleri; osteoporoz; polen; menopoz sonrası; arı sütü.

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- *This study was reported as oral presentation at the 10th Osteoporosis Osteoarthritis Joint Surgery Congress, April 20-24, 2012, Antalya, Turkey.*

Postmenopausal osteoporosis has become a major problem with significant morbidity and mortality.[1,2] Royal jelly (RJ) is the principal food of the honeybee queen. This is produced in the hypopharyngeal and mandibular glands of worker honeybees and has been demonstrated to possess several pharmacological activities such as life-span elongating,[3] antifatigue,[4] antiallergic,[5] antitumor,[6] antihypercholesterolemic,[7] antihypertensive,[8] and anti-inflammatory effects.[9] The chemical composition of RJ was reported to be proteins, sugars, lipids and vitamins.[10] It also contains many bioactive substances such as 10-hydroxy-2-decanoic acid and antibacterial protein.[4,5,10-12] It was also reported that RJ enhances intestinal absorption of calcium (Ca).[13] Bee pollen (BP) grains are small male reproduction units which are formed in the anthers of flowered plants, and collected by honeybees. Bee pollen contains organic materials such as amino acids, nucleic acids, enzymes, vitamins and hormones in their structures.[14] During ancient times, people throughout the world used pollen for its medical properties to alleviate or cure conditions such as colds, flu, ulcers, premature ageing, anemia, and colitis.[15]

The aim of this study was to investigate if royal jelly and bee pollen decrease bone loss due to osteoporosis in an oophorectomized rat model.

MATERIALS AND METHODS

Thirty-two female Sprague-Dawley mature rats at six-months-old, weighing 180-260 g were used for this study. The animals were fed ad libitum and housed in pairs in steel cages having a temperature-controlled environment (22±2 °C) with 12-h light/dark cycles. The animals were used with approval from the Experimental Animals Local Ethics Committee of the Erciyes University Medical Faculty. The study was conducted at Hakan Çetinsaya Clinical and Experimental Animals Research Center of the Erciyes University Medical Faculty. Animal care and experimental procedures were conducted in accordance with institutional guidelines[16] and animal rights preserved. Rats were divided into four groups, and each group consisted of eight rats. Group 1 was the sham-operation group. This group was considered as a control group, and all the surgical procedures were carried out except oophorectomy which was performed in the other groups. Group 2 had only oophorectomy. Group 3 had oophorectomy and the rats were fed by gastric lavage with RJ, 50 mg/kg body weight per day. Group 4 had oophorectomy and the rats were fed by gastric lavage with BP, 50 mg/kg body weight per day. Royal jelly and BP samples used in this study were obtained from the Civan Bee Farm in Bursa, Turkey. Effective doses were selected and used for RJ and BP according to data in the literature.[17,18]

All rats were anesthetized using ketamine hydrochloride at a dose of 50 mg/kg (Ketalar®, Eczacıbaşı), and 10 mg/kg of xylazine hydrochloride (Rompun®, Bayer) which were administered intraperitoneally.

Laparotomy was performed with ventral incision and reached to the ovariums. In group 1, only ovariums were explored and no surgical intervention was carried out. In the other groups bilateral oophorectomies were performed. Abdominal layers were closed anatomically, using 4-0 Vicryl® and the animals were allowed to recover from anesthesia.

The rats were followed for twelve weeks postoperatively to allow the development of moderate to severe osteoporosis. At the end of the study bone mineral density (BMD) of total body, lumbar spine and proximal left femur were measured using dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500 ELITE, Hologic Inc., Bedford, Massachusetts, U.S. X-Ray Bone Densitometer). Blood samples were taken from the tail vein to assess the levels of serum Ca, phosphate (P), and alkaline phosphatase (ALP) in all groups. Then, the rats were sacrified by cervical dislocation under intraperitoneally administered 60 mg/kg ketamine hydrochloride (Ketalar®, Eczacıbaşı, İstanbul, Turkey) anesthesia. The uterus of each rat was dissected and weighed at the end of sacrifice. The lumbar fourth vertebra body and left femoral head were dissected from each animal to make a standard evaluation for electron microscopic analysis. Longitudinal sections of tissue samples were taken under routine dehydration procedure at Erciyes University, Histology and Embryology Laboratory. After dehydration and the critical point dryer procedures, tissue samples were examined under scanning electron microscopy (SEM) (LEO 440, Electron Microscopy Ltd, Cambridge, UK) at 20 kV and images were taken at Erciyes University Technology Research and Developing Center. SEM-Energy Dispersive X-ray Spectrometry (SEM-EDX) was used to measure the Ca, P percentages and the ratio of Ca%/P% by point location in both lumbar vertebra and femoral head.

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Normality of the data was checked using the Kolmogorov–Smirnov test. The distribution was defined as means ± standard deviation (SD) because of the normal distribution of data. The difference among groups was determined by one way ANOVA test. Scheffe procedure was performed for the different group. Significance was set at $p<0.05$.

RESULTS

Uterine weights of rats in the sham-operation group were higher than the other groups which had oophorectomy. This difference among groups was statistically significant (p=0.001).

Total body BMD results indicated close values in all groups. There was no statistically significant difference among groups in terms of total body BMD (group 1; 0.153±0.009 gr/cm^2, group 2; 0.145±0.007 gr/cm^2, group 3; 0.145±0.007 gr/cm^2, group 4; 0.148±0.008 gr/cm^2) (p=0.19).

Both the lumbar spine and proximal femur BMD results indicated the highest values in group 1 (lumbar; 0.179±0.013 g/cm^2, femur; 0.160±0.011 g/cm^2), whereas the lowest values were obtained in group 2 (lumbar; 0.136±0.017 g/cm^2, femur; 0.119±0.005 g/cm^2). Although not as high as the values in group 1, it was found that group 3 (lumbar; 0.162±0.010 g/cm^2, femur; 0.143±0.010 gr/cm^2) and group 4 (lumbar; 0.161±0.017 g/cm^2, femur; 0.140±0.010 gr/cm^2) indicated high values in comparison with group 2. Statistically significant differences were found among groups in terms of BMD of both lumbar spine and proximal femur (p=0.001). In group 3 and group 4, the BMD results of the lumbar spine preserved their values when compared with group 1. However, they did not preserve their BMD values when compared with group 1 in terms of the BMD of the proximal femur. Nevertheless, in terms of the BMD of the proximal femur, group 3 and group 4 had statistically significant BMD values when compared with group 2 (p=0.001).

Although serum Ca levels were high in group 1 (10.30±1.02 mmol/L), the levels were close to each other in all groups (group 2; 9.57±1.44 mmol/L, group 3; 9.52±0.44 mmol/L, group 4; 9.76±0.71 mmol/L). The serum Ca levels were not statistically different among the groups (p=0.38).

Serum P levels were higher in group 2 (11.26±2.16 mmol/L) than the other groups. This difference was statistically significant when compared with group 1 (6.72±2.16 mmol/L), group 3 (7.76±1.79 mmol/L), and group 4 (7.86±2.16 mmol/L) (p=0.001).

Serum ALP levels were high in group 2 (308±47 IU/L) and group 4 (309±90 IU/L). This difference was statistically significant compared to group 1 (195±60 IU/L) (p=0.003).

Calcium%/P% ratios were high in group 4 (2.54±0.44) in terms of vertebra values with the analysis of SEM-EDX. Group 1 (2.36±0.31) and group 3 (2.35±0.37) had similar values of Ca%/P% ratio. Nevertheless, it was determined that these two groups had higher values than group 2 (2.00±0.05), and lower values than group 4. According to these values, a statistically significant difference was found between group 2 and group 4 (p=0.02). Although not statistically significant, in terms of Ca%/P% ratio of femoral head values with the analysis of SEM-EDX, the values in group 3 (2.44±0.48) and group 4 (2.44±0.60) were higher than group 1 (2.15±0.19) and group 2 (2.04±0.09) (p=0.12).

The images that were obtained by SEM analysis of lumbar vertebra and femoral head indicated significant cancellous bone loss with reduction of the number of trabeculae in group 2. On the other hand, it was seen that group 3 and 4 preserved their structure of trabeculae close to group 1 (Figures 1, 2).

DISCUSSION

Both postmenopausal women and oophorectomized rats lose bone as a result of ovarian hormone deficiency. The characteristics of bone loss in the rat model share similarities with those of early postmenopausal bone loss in many respects. The three-week-old rats are immature, and they have enormous bone remodelling. Twenty-four-month-old rats are anovulatory, and have increased incidence of diseases so that appropriate ages for an oophorectomized bone loss model are between these two ages.[19,20]

Marked atrophy of the uterus has been used as evidence of effectiveness of oophorectomy, because estrogen directly influences uterine weight. Oophorectomy results in a dramatic decrease in uterine weight.[11] In our study the uterine weights were significantly higher in the sham-operation group than the others.

In the mature rat model oophorectomy resulted in increased bone turnover that led to greater resorption than formation of cancellous bone, in agreement with both the aged rat model and female postmenopausal bone loss.[21] In our study there was no statistically significant difference in terms of total body BMD among all groups. But the results were statistically significant in terms of lumbar spine and proximal femur BMD of all groups. Accordingly, when the lumbar spine BMD values of the RJ and BP groups were compared with the sham-operation group, it was evident that they obtained a close value to the sham-operation group. Nevertheless, the difference was statistically significant only in the oophorectomy group when compared with the other groups. As for the proximal

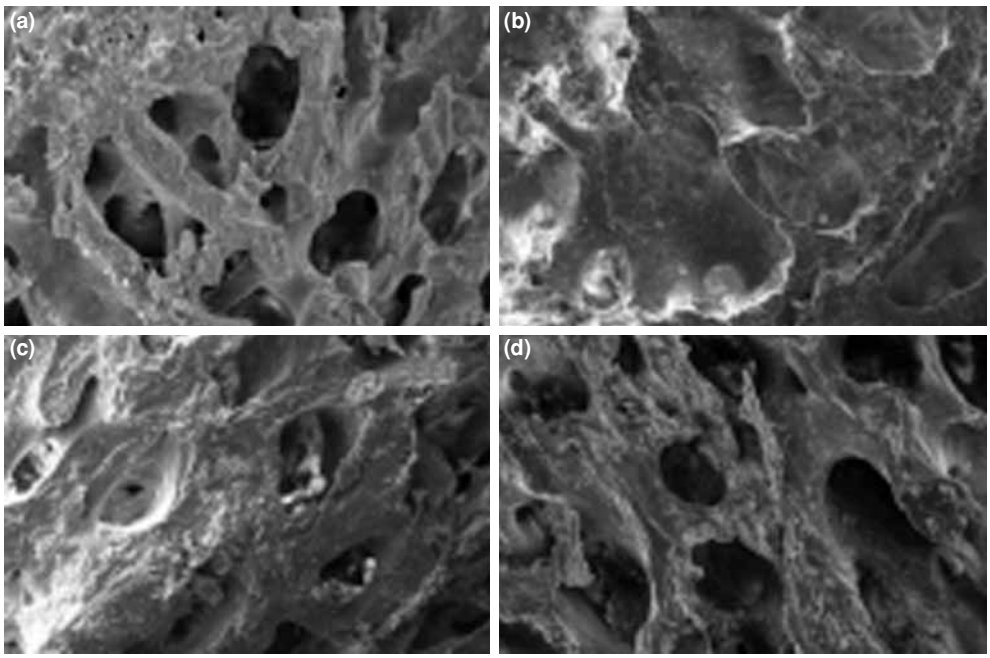

Figure 1. Scanning electron microscopy images of femoral heads. **(a)** Sham-operation group. **(b)** Oophorectomy group. **(c)** Royal jelly group. **(d)** Bee pollen group.

femur BMD values, RJ and BP groups were statistically significant when compared with the oophorectomy only group. The bone loss was lower in the RJ and BP groups than the oophorectomy group according to the BMD values of lumbar spine and proximal femur. In our study, BMD values of the proximal femur were lower than lumbar BMD values. Although the BMD of proximal femur values of the RJ and BP groups were not close to sham-operation group, they had higher values and statistically significant better results than the oophorectomy group. According to these results, RJ and BP were able to preserve BMD values, and they might be considered effective agents against bone loss that occurs because of estrogen deficiency.

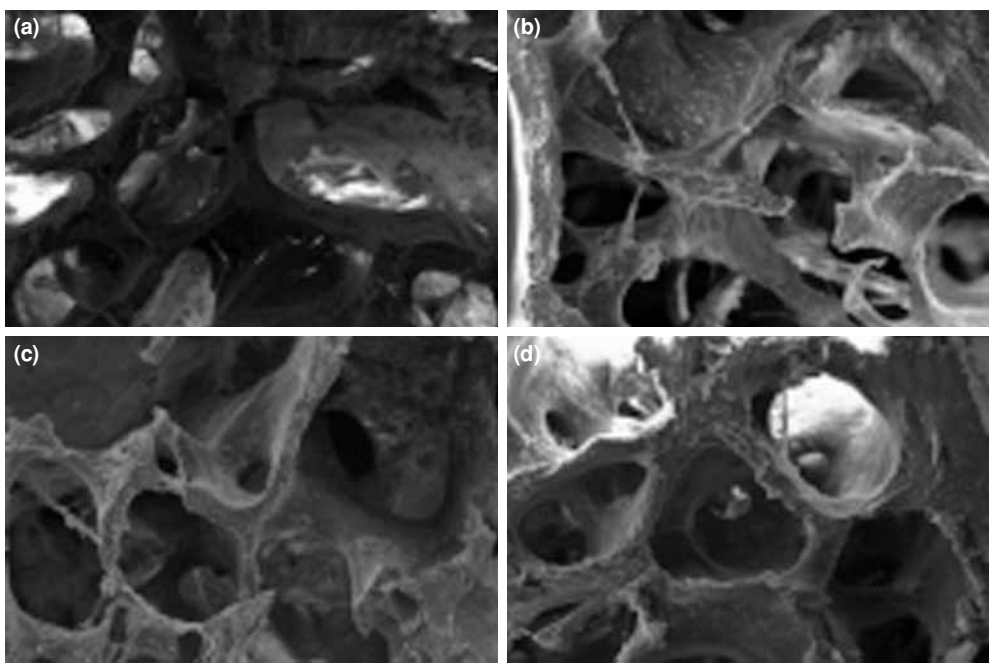

Figure 2. Scanning electron microscopy images of lumbar vertebras. **(a)** Sham-operation group. **(b)** Oophorectomy group. **(c)** Royal jelly group. **(d)** Bee pollen group.

Although a slight increase in serum Ca levels were reported early in menopause, both female postmenopausal bone loss and bone loss in the mature oophorectomized rat model are not associated with marked deviations of serum Ca levels from the normal range.[22] It was reported that following oophorectomy, total serum Ca, blood ionized Ca, and serum P levels are unchanged, but serum ALP levels are increased conversely.[23] In our study, statistically significant difference was not found for Ca levels among all groups, but serum ALP and P levels were found statistically significant.

A decrease in BMD values may be due to a decrease in either Ca or P, or dissimilar decreases in both. As a result, the determination of Ca/P ratio may provide a sensitive measure of bone mineral changes and may add to our understanding of the changes occurring in bone diseases.[24] Also it was reported that SEM-EDX analysis was a useful semiquantitative tool for evaluating bone mineral composition and determining Ca and P rates in bone structure.[25] Osteoporotic females indicate significantly lower Ca/P ratio than perimenopausal females.[26] In our study, Ca%/P% ratios were found close to each other for all groups except the oophorectomy group by SEM-EDX analysis of lumbar vertebra. A statistically significant difference was found between oophorectomy group and BP group. Although there was no statistically significant difference between the groups in the femoral head, the Ca%/P% ratios were highest in the RJ and BP groups, and lowest in the oophorectomy only group. In terms of both the lumbar vertebra and femoral head, the RJ and BP groups were able to preserve their Ca%/P% ratios in contrast to expected decrease when compared with the oophorectomy group. According to these findings, RJ and BP may be considered to have beneficial effects on bone mineral changes of osteoporotic bone. Although the number of endosteal osteoclasts increase in the mature oophorectomized rat model, cortical bone does not decrease significantly, in spite of a dramatic decrease in metaphyseal cancellous bone. This finding is in line with the greater loss of cancellous than cortical bone in postmenopausal women. In our study, it was evident with the images of SEM that the RJ and BP groups preserved their structure of trabeculae as nearly as the sham-operation group. Therefore, RJ and BP may be considered to be preservative for the structure and the number of trabeculae.

Although some molecules increase BMD much more than others, all of them decrease the fracture risk at similar rates.[27] Accordingly, natural products without side effects like bee products, such as RJ or BP can be used alternatively for the treatment of osteoporosis and they may also reduce the osteoporotic fracture risk. Royal jelly also contains a male hormone testosterone. Because of this feature it may also be effective in men's osteoporosis that could be induced by a decrease of androgen.[11,28] Considering all these features, RJ and BP may be very beneficial in the treatment of osteoporosis.

Declaration of conflicting interests

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REFERENCES

1. Atik OS, Gunal I, Korkusuz F. Burden of osteoporosis. Clin Orthop Relat Res 2006;43:19-24.
2. Atik OS. Is DXA the gold standard? Eklem Hastalik Cerrahisi 2011;22:63.
3. Inoue S, Koya-Miyata S, Ushio S, Iwaki K, Ikeda M, Kurimoto M. Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. Exp Gerontol 2003;38;965-9.
4. Kamakura M, Mitani N, Fukuda T, Fukushima M. Antifatigue effect of fresh royal jelly in mice. J Nutr Sci Vitaminol 2001;47;394-401.
5. Okamoto I, Taniguchi Y, Kunikita T, Kohno K, Iwaki K, Ikeda M, et al. Major royal jelly protein 3 modulates immune responses in vitro and in vivo. Life Sci 2003;1:2029-45.
6. Bincoletto C, Eberlin S, Figueiredo CA, Luengo MB, Queiroz ML. Effects produced by royal jelly on haematopoiesis: relation with host resistance against Ehrlich ascites tumour challenge. Int Immunopharmacol 2005;5:679-88.
7. Vittek J. Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. Experientia 1995;51:927-35.
8. Matsui T, Yukiyoshi A, Doi S, Sugimoto H, Yamada H, Matsumoto K. Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. J Nutr Biochem 2002;13:80-6.
9. Fujii A, Kobayashi S, Kuboyama N, Furukawa Y, Kaneko Y, Ishihama S, et al. Augmentation of wound healing by royal jelly (RJ) in streptozotocindiabetic rats. Jpn J Pharmacol 1990;53:331-7.
10. Takenaka T. Chemical composition of royal jelly. Honeybee Sci 1982;3:69-74.
11. Hidaka S, Okamoto Y, Nakajima K, Suekawa M, Liu SY. Preventive effects of traditional Chinese (Kampo) medicines on experimental osteoporosis induced by ovariectomy in rats. Calcif Tissue Int 1997;61:239-46.
12. Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. Apotent antibacterial protein in royal jelly. J Biol Chem 1990;265:11333-7.
13. Nakasa T, Okinaka O, Nakatsuka M. Promotion effect of calcium absorption by protease-treated royal jelly. Food Dev 1999;34:42-4.

14. Orzáez Villanueva MT, Díaz Marquina A, Bravo Serrano R, Blazquez Abellán G. The importance of bee-collected pollen in the diet: a study of its composition. International Journal of Food Sciences and Nutrition 2002;53:217-24.
15. Hanssen M. The healing power of polen and other products from the beehive: propolis, royal jelly, honey. Wellingborough, Northamptonshire: Thorsons; 1979.
16. Available from: http://www.nap.edu/catalog/5140.html
17. Kanbur M, Eraslan G, Silici S, Karabacak M. Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters. Food and Chemical Toxicology 2009;47:1184-89.
18. Eraslan G, Kanbur M, Silici S. Effect of carbaryl on some biochemical changes in rats: The ameliorative effect of bee pollen. Food and Chemical Toxicology 2009;47:86-91.
19. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Miner 1991;15:175-91.
20. Wronski TJ, Yen CF. The ovariectomized rat animal model for postmenopausal bone loss. Cells Mater Suppl 1991;1:69-74.
21. Wronski TJ, Dann LM, Scott KS, Cintron M. Long-term effects of ovariectomy and aging on the rat skeleton. Calcif Tiss Int 1989;45:360-6.
22. Fogelman I, Poser JW, Smith ML, Hart DM, Bevan JA. Alterations in skeleton metabolism following oophorectomy. Osteoporosis 1984;519-22.
23. Kalu DN, Echon R, Hollis BW. Modulation of ovariectomy-related bone loss by parathyroid hormone in rats. Mech Ageing Dev 1990;56:49-62.
24. Mazess RB, Peppler WW, Chesnut CH 3rd, Nelp WB, Cohn SH, Zanzi I. Total body bone mineral and lean body mass by dual-photon absorptiometry. II. Comparison with total body calcium by neutron activation analysis. Calcif Tissue Int 1981;33:361-3.
25. Akesson K, Grynpas MD, Hancock RG, Odselius R, Obrant KJ. Energy-dispersive X-ray microanalysis of the bone mineral content in human trabecular bone: a comparison with ICPES and neutron activation analysis. Calcif Tissue Int 1994;55:236-9.
26. Fountos G, Tzaphlidou M, Kounadi E, Glaros D. In vivo measurement of radius calcium/phosphorus ratio by X-ray absorptiometry. Appl Radiat Isot 1999;51:273-8.
27. Atik OS. Osteoporotic fracture risk assessment. Eklem Hastalik Cerrahisi 2008;19:100.
28. Vittek J, Slomiany BL. Testosterone in royal jelly. Experientia 1984;40:104-6.