Original Article

Mahidol University Journal of Pharmaceutical Sciences 2014; 41 (4), 29-38

Effects of Antiresorptive Drugs on Long Bone Growth, Body Weight Gain, and Serum Lipid Levels in Young Adult Ovariectomized Rats

N. Sookvanichsilp, N. Tad-urai, P. Lawtong and G. Sittithumcharee*
Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

Abstract

Although osteoporosis is common among the female elderly, in young adults who are associated with breast cancer treatment or under ovarian abrasion are also at risk to low bone mass. The present study aimed to investigate the benefits in preventing bone deterioration due to estrogen deficiency of antiresorptive drugs, i.e. conjugated equine estrogens (CEE), alendronate and raloxifene in young adult ovariectomized (OVX) rats. Effects of the drugs on body weight gain, serum total cholesterol and serum triglyceride levels were also investigated. Nine-week old female rats were treated orally on the day of ovariectomy with 3 mg/kg/day alendronate, 0.1 mg/kg/day CCE, or 3 mg/kg/day raloxifene for 8 weeks. The results demonstrated that ovariectomy caused a decrease in the growth of long bones as evaluated by the weight, length, volume and calcium content of the femurs and tibias. Ovariectomy also caused an increase in body weight gain and serum total cholesterol while it did not cause any changes in serum triglyceride. Alendronate and CEE, but not raloxifene could effectively prevent bone deterioration due to estrogen deficiency in the OVX rats. Among the three drugs, only alendronate exhibited no significant effects on body weight gain and serum lipids of the OVX rats. Estrogen, although it could delay the body weight increment and had cholesterol-lowering effects, it caused an increase in serum triglyceride. The present results have suggested that in young adult females who are subjected to estrogen deprivation, CEE or alendronate may be effective in preventing the bone from deterioration. However, their adverse events should be taken into account in the long-term use.

Keyword: Antiresorptive drug, Ovariectomized rat, Bone growth, Body weight gain, Cholesterol, Triglyceride

INTRODUCTION

Osteoporosis is a progressive systemic disease characterized by a decrease in bone mineral density (BMD) and microarchitectural deterioration of bone tissue[1-3]. It is the most prevalent disease among postmenopausal women, resulting from a decrease in circulating estrogens. In young adults who do not achieve their ideal peak bone mass, they may develop osteoporosis at a much earlier age[4,5]. A recent study has shown that significant decrease in BMD is occurring in women aged 25-45 at several skeletal sites[6]. In addition, young adults who are associated with breast cancer treatment[7] or subjected to glucocorticoid treatment[4,7,8] or under ovarian abrasion[4,8] are also at risk to osteoporosis.

Antiresorptive agents (agents that block bone resorption by inhibiting the activity of osteoclasts) and anabolic agents (agents that stimulate bone formation by acting primarily on osteoblasts) including estrogens, raloxifene, alendronate, vitamin D, terparatide, and new biologics are available for the management of osteoporosis[1,3,4,9]. Among these, estrogens, raloxifene (a selective estrogen receptor modulator or a SERM) and alendronate (a well-known bisphosphonate) which are antiresorptive drugs are still commonly used.

***Corresponding author:** Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Bangkok 10400, Thailand. Fax: (662) 644-8700. E-mail: nongluck.soo@mahidol.ac.th

Several previous studies have focused on the benefits of antiresorptive drugs in increasing BMD in osteoporotic animal models[10-15] or in postmenopausal osteoporotic women[16-19] but data about their effects in the prevention of estrogen deficiency-related bone deterioration in young adults are limited. Therefore, the present study aimed to investigate the benefits in preventing bone deterioration due to estrogen deficiency of antiresorptive drugs, i.e. CEE, alendronate and raloxifene in young adult OVX rats.

Although the relationship between cardiovascular disease (CVD) and osteoporosis in the elderly has remained unclear, previous studies have demonstrated in women the association between CVD and osteoporosis severity[20], hip fracture[21], or low bone mass[22]. In addition, weight gain[13,23] and changes in serum lipid levels[24,25] can occur after menopause or estrogen deprivation. Benefits of antiresorptive drugs on these parameters in osteoporotic postmenopausal patients[17,26,27] or osteoporotic animal models[13,14,28] have been reported. Whether benefits of the antiresorptive drugs, CEE, alendronate and raloxifene in preventing changes of body weight, serum total cholesterol and serum triglyceride are also achieved in the young adults associated with estrogen deprivation were also investigated in the present study using young adult OVX rat model.

MATERIALS AND METHODS

Animals

Nine-week old young adult female Sprague-Dawley rats weighing between 190-225 g were obtained from the National Animal Center, Mahidol University at Salaya Campus, Nakorn Pathom Province, Thailand. They were housed in the air conditioning room with a 12-hour light-dark cycle. The animals were fast overnight before drug administration in the morning. All rats had free access to water. The experiment protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University.

Experimental design

Bilateral ovariectomy or sham operation was performed under ether anesthesia. The rats were randomly divided into 5 groups of 8-9 animals each, i.e. sham, OVX control and OVX rats treated orally with raloxifene (Celvista® 60 mg tablets, Elli Lilly) at a dose of 3 mg/kg/day, alendronate (Fosamax® 70 mg tablets, Merck Sharp & Dohme) at a dose of 3 mg/kg/day, or conjugated equine estrogens (CEE; Premarin® 1.25 mg tablets, Wyeth-Ayerst) at a dose of 0.1 mg/kg/day. The drug doses were taken from other studies regarding anti-osteoporotic purposes[29-32]. Treatment was given started on the day of ovariectomy and continued for 8 weeks. The body weight was checked weekly. Blood was collected from tail tips under ether anesthesia at a 2-week interval. At the end of treatment, the right femurs and tibias of all sacrificed rats were carefully removed for the subsequent determinations.

Determination of bone growth (weight, volume and length)

The right femur and tibia (with fibula) were dissected and cleaned off soft tissues. The bone length was measured using a Vernier caliper (Naza, China). To determine the bone volume, trapped air in bone was liberated by immersing the bone in an unstopped glass bottle filled with deionized water. The bottle was placed into a sealed plastic bag connected to a vacuum generator for 60 min so that trapped air would diffuse out of the bone. The bone volume was measured with a plethysmometer (Ugo Basile, Italy). The bone was then dried to a constant weight.

Determination of bone calcium content

Dried tibia and femur of each animal were cut into small pieces, combined and burned to ash at 600° C for 12 h in a muffle furnace (Lenton SAF11/1, UK). The calcium content was determined using a flame atomic absorption spectrophotometer (GBC 932 Plus, Australia). Briefly, about 0.0300 g of ash was accurately weighed and dissolved in 1 ml of 6 N HCl. A volume of 4 ml of 60 mg/ml lanthanum was then added and the solution was further diluted with deionized water to 25 ml in a volumetric flask. The

resulting solution was filtered through a Whatman No.1 filter paper and an adequate amount of the filtrate was submitted for calcium determination. A standard curve was conducted using different calcium solutions, 2-760 ppm.

Determination of serum total cholesterol and triglyceride levels

All rats were fasted over night before blood sampling. Blood was collected from tail tips under ether anesthesia at weeks 0, 2, 4, 6 and 8 of ovariectomy. Serum was obtained following blood centrifugation for 10 min at 1000 rpm. Serum total cholesterol and triglyceride levels were determined using commercial assay kits (CPT Diagnostics, Spain).

Data analysis

Data were analyzed by computerized programs and expressed as means ± SEM. Differences among groups were analyzed using one-way analysis of variance (ANOVA) for multiple comparisons. Statistical significance was determined using Dunnett's post hoc and the p-value of less than 0.05 indicated a significant difference between groups.

RESULTS

Effect on the body weight

Ovariectomy caused a significant increase in the mean body weight of young adult rats since week 2 till the end of the 8-week period (Table 1). CEE and raloxifene, but not alendronate, could delay (for CEE) or prevent (for raloxifene) the body weight increment.

Effect on the bone growth (weight, length, volume and calcium content)

Eight weeks of ovariectomy caused a decrease in the growth of long bones of young adult rats as evaluated by the weight, length, volume and calcium content of the femurs and tibias although significant decreases could be observed only for some parameters, i.e. bone length and calcium content (Table 2). Treatment with CEE or alendronate, but not raloxifene, could prevent the decrease in all those parameters. Moreover, alendronate could increase all the parameters, compared with sham rats, although significant differences could be observed only for tibia weight and calcium content. Compared with CEE, alendronate seemed to be more effective in promoting bone growth and it could also produce a significantly higher increase in bone weight and calcium content.

Effects on serum total cholesterol and triglyceride

Ovariectomy caused an increase in serum total cholesterol levels in young adult rats and significant changes were found compared with sham rats since week 2 (Table 3). CEE and raloxifene, but not alendronate, could prevent the increase in serum total cholesterol due to ovariectomy. In addition, they could significantly reduce serum total cholesterol when compared with the baseline or with the levels in sham group at the corresponding time points.

Serum triglyceride levels seemed to be gradually increased from the baseline in all groups and the increases were significant at week 8 of treatment (Table 4). Ovariectomy, however, did not cause any significant changes in serum triglyceride levels of young adult rats compared with sham rats. Alendronate or raloxifene had no significant effect on changes in serum triglyceride levels while CEE significantly increased the levels, starting from week 6, either compared with OVX or sham rats.

DISCUSSION

Body weight and fat mass tend to increase with menopause[23]. In the present study, as expected, there was a significant increase in the body weight of the young adult rats since week 2 post-ovariectomy. In other studies, regardless of animal age, ovariectomy also caused a significantly higher increase in body weight gain compared with non-OVX rats or sham rats[13,30,33,34]. The results of estrogen (CEE) and raloxifene which could attenuate the body weight gain

Table 1. Effects of antiresorptive drugs on the body weight (g) of young adult ovariectomized (OVX) rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	% Increasea
Sham	203.25 ± 2.37	221.63 ± 2.74	236.06 ± 4.25*	245.81 ± 4.62**	251.50 ± 5.22**	262.88 ± 5.64**	266.56 ± 5.65**	272.75 ± 6.52**	271.13 ± 6.20**	33.54 ± 3.56**
OVX control	203.50 ± 2.90	228.69 ± 3.92	259.63 ± 5.77	291.25 ± 6.04	307.44 ± 6.91	317.31 ± 7.25	323.50 ± 8.61	327.50 ± 12.08	334.69 ± 11.50	64.81 ± 6.70
Alendronate, 3 mg/kg/day	201.22 ± 2.13	228.78 ± 2.90	267.28 ± 3.40††	292.83 ± 4.11††	314.22 ± 4.85††	324.44 ± 5.46††	335.28 ± 6.23††	339.78 ± 5.91††	342.33 ± 7.71††	70.22 ± 4.00††
CEE, 0.1 mg/kg/day	204.94 ± 2.28	225.28 ± 4.24	251.44 ± 4.19	270.83 ± 6.07*†	288.39 ± 6.51††	299.28 ± 5.97††	311.00 ± 5.81††	315.00 ± 7.29†	315.39 ± 7.90†	53.84 ± 3.16†
Raloxifene, 3 mg/kg/day	203.83 ± 2.51	215.74 ± 3.32*	236.00 ± 3.90*	244.89 ± 5.03**	256.44 ± 5.09**	257.22 ± 3.60**	266.22 ± 5.42**	262.44 ± 8.06**	267.94 ± 6.95**	31.39 ± 2.67**

Values are means ± SEM. There were 8-9 rats per group.
CEE = conjugated equine estrogens.
[a] = $\frac{\text{(body weight at week 8 – body weight at week 0)}}{\text{body weight at week 0}} \times 100$
Significant difference from OVX group at corresponding time: * $p < 0.05$, ** $p < 0.001$.
Significant difference from sham group at corresponding time: † $p < 0.05$, †† $p < 0.001$.

Table 2. Effects of antiresorptive drugs on the growth of the femur and tibia of young adult ovariectomized (OVX) rats

Treatment	Weight (g)		Volume (ml)		Length (cm)		Total Ca (mg)
	Femur	Tibia	Femur	Tibia	Femur	Tibia	Femur + Tibia
Sham	0.458 ± 0.009	0.360 ± 0.005	0.380 ± 0.008	0.289 ± 0.011	3.313 ± 0.017**	3.765 ± 0.020*	213.704 ± 5.493*
OVX control	0.431 ± 0.009	0.340 ± 0.011	0.353 ± 0.008	0.275 ± 0.011	3.194 ± 0.018	3.639 ± 0.030	193.387 ± 5.049
Alendronate, 3 mg/kg/day	0.486 ± .007**	0.405 ± 0.010**†	0.389 ± 0.008*	0.314 ± 0.010*	3.316 ± 0.029**	3.806 ± 0.027**	235.159 ± 5.076**†
CEE, 0.1 mg/kg/day	0.452 ± 0.007 $^{+}$	0.353 ± 0.007^{++}	0.379 ± 0.010	0.291 ± 0.009	3.298 ± 0.021*	3.738 ± 0.027*	208.445 ± 4.355^{+}
Raloxifene, 3 mg/kg/day	0.427 ± 0.008	0.337 ± 0.006	0.348 ± 0.006†	0.269 ± 0.005	3.186 ± 0.015††	3.620 ± 0.020††	190.809 ± 5.222

Values are means ± SEM. There were 8-9 rats per group.
CEE = conjugated equine estrogens.
Significant difference from OVX group: * $p < 0.05$, ** $p < 0.001$.
Significant difference from sham group: † $p < 0.05$, †† $p < 0.001$.
Significant difference from alendronate group (compared with CEE group only): $^{+}$ $p < 0.05$, $^{++}$ $p < 0.001$.

Table 3. Effects of antiresorptive drugs on serum cholesterol levels (mg/dl) in young adult ovariectomized (OVX) rats

Treatment	Week 0	Week 2	Week 4	Week 6	Week 8
Sham	101.65 ± 2.52	105.82 ± 2.17**	104.34 ± 2.37**	107.81 ± 3.01*	108.07 ± 2.57*
OVX control	99.61 ± 3.72	125.32 ± 3.78$^{\#}$	126.93 ± 2.14$^{\#\#}$	128.24 ± 4.71$^{\#}$	131.24 ± 2.94$^{\#\#}$
Alendronate, 3 mg/kg/day	101.06 ± 2.27	111.07 ± 4.53	123.74 ± 5.61$^{\#}$	122.82 ± 3.07$^{\dagger\#}$	125.99 ± 3.72$^{\dagger\#\#}$
CEE, 0.1 mg/kg/day	104.33 ± 4.33	95.18 ± 2.15**†	89.02 ± 1.89$^{**\dagger\#}$	92.01 ± 2.04**†	86.69 ± 2.51$^{**\dagger\dagger\#\#}$
Raloxifene, 3 mg/kg/day	99.44 ± 2.37	97.86 ± 2.18**	90.35 ± 2.50$^{**\dagger\#}$	90.79 ± 2.12$^{**\dagger\#}$	84.44 ± 2.01$^{**\dagger\dagger\#}$

Values are means ± SEM. There were 8-9 rats per group.
CEE = conjugated equine estrogens.
Significant difference from OVX group at corresponding time: * $p < 0.05$, ** $p < 0.001$.
Significant difference from sham group at corresponding time: † $p < 0.05$, †† $p < 0.001$.
Significant difference from week 0: $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.001$.

Table 4. Effects of antiresorptive drugs on serum triglyceride levels (mg/dl) in young adult ovariectomized (OVX) rats

Treatment	Week 0	Week 2	Week4	Week 6	Week 8
Sham	77.67 ± 3.71	77.41 ± 2.30	81.49 ± 3.27	84.01 ± 2.01	92.00 ± 2.14$^{\#}$
OVX control	76.25 ± 2.89	82.46 ± 4.94	90.94 ± 3.83$^{\#}$	89.78 ± 2.04$^{\#}$	95.04 ± 1.96$^{\#}$
Alendronate, 3 mg/kg/day	70.40 ± 3.31	76.49 ± 4.43	83.31 ± 1.89$^{\#}$	82.17 ± 2.65$^{\#}$	84.67 ± 2.50$^{\#}$
CEE, 0.1 mg/kg/day	73.45 ± 4.25	78.51 ± 4.02	85.98 ± 2.35$^{\#}$	106.12 ± 2.08$^{*\dagger\dagger\#\#}$	113.07 ± 3.13$^{*\dagger\dagger\#\#}$
Raloxifene, 3 mg/kg/day	74.03 ± 3.26	75.93 ± 3.25	80.51 ± 3.26	86.59 ± 2.68$^{\#}$	88.27 ± 3.21$^{\#}$

Values are means ± SEM. There were 8-9 rats per group.
CEE = conjugated equine estrogens.
Significant difference from OVX group at corresponding time: * $p < 0.05$.
Significant difference from sham group at corresponding time: † $p < 0.05$, †† $p < 0.001$.
Significant difference from week 0: $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.001$.

in young adult OVX rats in the present study were in agreement with previous studies which showed that treatment with either estrogen[13,30] or raloxifene[13,14,30] could reduce body weight increment of OVX rats to nearly equal to sham rats. On the other hand, alendronate in the present study had no effect on the increment of body weight. Recent studies also showed that alendronate could not prevent the body weight gain of OVX rats[30,33]. As seen in many studies including the present one, the body weight of animals is gradually increased after ovariectomy and antiresorptive drugs may have different effects on the body weight gain. The results can be therefore distorted if the data are expressed in terms based on the final body weight.

Bone growth, in humans as well as in animals, includes the progressive incremental changes in length, size and mass. Female rats usually stop growth at age 85-100 days but the epiphysis, however, never completely closes[35]. The process of bone aging in rats has begun at the age around 40-50 weeks[36] and to support this, in the present study weight of the femurs and tibias of sham rats at age 17 weeks (on the day of sacrifice) were lower than those at age 25 weeks[37]. Sex steroids are known to regulate skeletal growth and maturation in both men and women[3,38] and also in animals[39]. Many studies have revealed deterioration to bone (evaluated by the decrease in BMD, bone weight, or calcium content) in animals, regardless of animal age, after ovariectomy[33,37,40] and treatment with estrogens could correct or delay bone deterioration due to estrogen deprivation[37,40]. To support the role of sex steroids in regulating skeletal growth in young adult animals, treatment with CEE in the present study could preserve normal bone growth as evaluated by the weight, length, volume and calcium content of the femur and tibia. Since rats, unlike humans, do not undergo epiphyseal fusion[41,42], they are probably appropriate for studying the effect of drugs on bone elongation when the effect from epiphyseal closure is excluded. The present study has therefore demonstrated that estrogens do have the role in enhancing longitudinal bone growth when the process of bone aging is not reached. Raloxifene, unlike estrogen, although it promotes bone antiresorptive activity in aging OVX rats[10,11] and in osteoporotic postmenopausal women[43,44] where ovarian estrogens are depleted, it is generally known that SERMs should be avoided in menstruating women as they can cause further bone loss[4]. In addition, previous studies showed that raloxifene inhibited longitudinal bone elongation in rats without the effect to hasten epiphyseal fusion[31,45]. Together with the result of the present study, raloxifene may not be appropriate for preserving normal bone growth in young adults associated with estrogen deprivation.

Although many studies could confirm the preventive effect of alendronate on the bone loss induced by estrogen deficiency in adult OVX rats[29,33], the results of alendronate on bone growth are limited. OVX rats treated with alendronate orally at the same dose as in the present study but only for 4 weeks could not cause any significant changes in the length and diameter of the femur[29]. Long-term treatment with other bisphosphonates, ibandronate and risedronate, for 4 months dose-dependently increased the volume of vertebral trabecular and cortical bone and also tibia cortical bone of aging rats (2 years old)[46]. Alendronate treatment in children with glucocorticoid-induced osteoporosis could inhibit the loss of long bones[47]. Since alendronate can bind to hydroxyapatite during the process of mineralization which becomes buried by new bone formation[9,48] and it also promotes osteoclast apoptosis[9,49], therefore taken together, these could be the reasons why treatment with alendronate in the present study could overcome the decrease in bone growth after ovariectomy in young adult rats when the process of bone aging is not reached.

In postmenopausal women[24,25,27] as well as in OVX rats[13,14,34], estrogen depletion consistently causes an increase in cholesterol levels. Treatment with CCE and raloxifene, but not alendronate, could effectively prevent the increase in serum cholesterol throughout

the present study which was consistent with the results from many previous studies in OVX rats regardless of animal age when treated with estrogens[13,30,50] or raloxifene[13,30,51] or alendronate[28,30]. In postmenopausal women, estrogens or estrogen plus progestin[52-54] as well as raloxifene[27,52,55] could also reduce total cholesterol and/or low density lipoprotein cholesterol (LDL-C) while alendronate again could not cause any significant changes in serum total cholesterol in these subjects[19,27].

Estrogen deprivation due to ovariectomy did not cause significant changes in serum triglyceride in the present study as well as in many other studies which the investigations were performed at different periods after ovariectomy[12,34,51]. Previous studies have suggested that oral estrogens may influence plasma triglyceride by their actions on hepatic synthesis and release of triglyceride[56,57]. Indeed, treatment with estrogen in postmenopausal women (estrogen alone or in combination with a progestin)[24,50,53] as well as in OVX rats (estrogen alone)[58,59] including also in young adult rats in the present study, could increase serum triglyceride levels. Furthermore, some studies revealed that oral estrogens could increase serum triglyceride levels in a dose dependent manner[60,61]. Raloxifene and alendronate had neutral effects on serum triglyceride as found in the present study and other studies in OVX rats [12,51,58] as well as in postmenopausal women[19,27,55].

In conclusion, CEE as well as alendronate provides not only the benefit on BMD in osteoporotic subjects as revealed by many previous studies, but may also have the benefits in preventing bone deterioration due to estrogen deficiency in the young adults. Moreover, they demonstrated no undesirable effects on body weight gain and serum total cholesterol levels. Estrogen, although it has cholesterol-lowering effects, it can cause an increase in serum triglyceride levels. On the other hand, although raloxifene provides the benefit on BMD in osteoporotic subjects as revealed by many previous studies and it also demonstrated no undesirable effects on body weight gain, serum total cholesterol and triglyceride levels, it may have no benefits in preventing bone deterioration due to estrogen deprivation in the young adults. Taken together, the present results have suggested that among the three antiresorptive drugs (CEE, alendronate and raloxifene), CEE and alendronate as a monotherapy may be appropriate for the prevention of bone deterioration in the young adult females who are associated with estrogen deprivation. However, their particular potential adverse effects, i.e. gastro-esophageal ulcers from alendronate, increased risk of endometrial and breast cancers and also some CVS adverse events from estrogen should be taken into account especially in the long-term use.

ACKNOWLEDGEMENTS

This study was supported by a grant from Mahidol University, Bangkok, Thailand.

REFERENCES

1. Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol* 2014; 142: 155-70.
2. Mazziotti G, Bilezikian J, Canalis E, *et al.* New understanding and treatments for osteoporosis. *Endocrine* 2012; 41: 58-69.
3. Moseley KF, De Beur SMJ. Osteoporosis in men and women. In: Legato MJ, editor. Principles of gender-specific medicine, 2nd edition. London: Academic Press, 2010:716-36.
4. Bhalla AK. Management of osteoporosis in a pre-menopausal woman. *Best Pract Res Clin Rheumatol* 2010; 24:313-27.
5. Riggs BL, Melton LJ, Robb RA, *et al.* A population based assessment of rates of bone loss at multiple skeletal sites: evidence for substantial trabecular bone loss in young adult women and men. *J Bone Miner Res* 2008; 23:205-14.
6. Shaffer JR, Kammerer CM, Dressen AS, *et al.* Rate of bone loss is greater in young Mexican American men than women: The San Antonio Family Osteoporosis Study. *Bone* 2010; 47:49-54.
7. Mazziotti G, Canalis E, Giustina A. Drug-induced osteoporosis: mechanisms and clinical implications. *Am J Med* 2010; 123; 877-84.

8. North American Menopause Society. Management of osteoporosis in postmenopausal women: 2010 position statement of The North American Menopause Society. *Menopause* 2010; 17:25-54.
9. Reginster J-Y. Antifracture efficacy of currently available therapies for postmenopausal osteoporosis. *Drugs* 2011; 71:65-78.
10. Martel C, Picard S, Richard V, *et al.* Prevention of bone loss by EM-800 and raloxifene in the ovariectomized rat. *J Steroid iochem Mol Biol* 2000; 74:45-56.
11. Turner CH, Sato M, Bryant HU. Raloxifene preserves bone strength and bone mass in ovariectomized rats. *Endocrinol*ogy 1994; 135:2001-5.
12. Black LJ, Sato M, Rowley ER, *et al.* Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J Clin Invest* 1994; 93:63-9.
13. Sato M, Rippy MK, Bryant HU. Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats. *FASEB J* 1996; 10:905-12.
14. Sato M, Turner CH, Wang T, *et al.* LY353381.HCl: a novel raloxifene analog with improved SERM potency and efficacy *in vivo*. *J Pharmacol Exp Ther* 1998; 287:1-7.
15. Diab T, Wang J, Reinwald S, *et al.* Effects of the combination treatment of raloxifene and alendronate on the biomechanical properties of vertebral bone. *J Bone Miner Res* 2011; 26:270-6.
16. Reid DM, Hosking D, Kendler D, *et al.* A comparison of the effect of alendronate and risedronate on bone mineral density in postmenopausal women with osteoporosis: 24-month results from FACTS-international. *Int J Clin Pract* 2008; 62: 575-84.
17. Bone HG, Greenspan SL, McKeever C, *et al.* Alendronate and estrogen effects in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* 2000; 85:720-6.
18. Davas I, Altintas A, Yoldemir T, *et al.* Effect of daily hormone therapy and alendronate use on bone mineral density in postmenopausal women. *Fertil Steril* 2003; 80:536-40.
19. Sanad Z, Ellakwa H, Desouky B. Comparison of alendronate and raloxifene in postmenopausal women with osteoporosis. *Climacteric* 2011; 14: 369-77.
20. Tanko LB, Christiansen C, Cox DA, *et al.* Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res* 2005; 20:1912-20.
21. Sennerby U, Melhus H, Gedeborg R, *et al.* Cardiovascular diseases and risk of hip fracture. *JAMA* 2009; 302:1666-73.
22. Marcovitz PA, Tran HH, Franklin BA, *et al.* Usefulness of bone mineral density to predict significant coronary artery disease. *Am J Cardiol* 2005; 96:1059-63.
23. Ongphiphadhanakul B, Chanprasertyothin S, Piaseu N, *et al.* Change in body weight after hormone replacement therapy in postmenopausal women is dependent on basal circulating leptin. *Maturitas* 1998; 30:283-8.
24. Abbey M, Owen A, Suzakawa M, *et al.* Effects of menopause and hormone replacement therapy on plasma lipids, lipoproteins and LDL-receptor activity. *Maturitas* 1999; 33:259-69.
25. Graff-Iversen S, Thelle DS, Hammar N. Serum lipids, blood pressure and body weight around the age of the menopause. *Eur J Cardiovasc Prev Rehabil* 2008; 15:83-8.
26. Roussel AM, Bureau I, Favier M, *et al.* Beneficial effects of hormonal replacement therapy on chromium status and glucose and lipid metabolism in postmenopausal women. *Maturitas* 2002; 42:63-9.
27. Iwamoto J, Sato Y, Uzawa M, *et al.* Comparison of effects of alendronate and raloxifene on lumbar bone mineral density, bone turnover, and lipid metabolism in elderly women with osteoporosis. *Yonsei Med J* 2008; 49:119-28.
28. Helvering LM, Liu R, Kulkarni NH, *et al.* Expression profiling of rat femur

revealed suppression of bone formation genes by treatment with alendronate and estrogen but not raloxifene. *Mol Pharmacol* 2005; 68:1225-38.
29. Sliwiński L, Janiec W, Pytlik M, *et al.* Effect of administration of alendronate sodium and retinol on the mechanical properties of the femur in ovariectomized rats. *Pol J Pharmacol* 2004; 56:817-24.
30. Frolik CA, Bryant HU, Black EC, *et al.* Time-dependent changes in biochemical bone markers and serum cholesterol in ovariectomized rats: effects of raloxifene HCl, tamoxifen, estrogen, and alendronate. *Bone* 1996; 18:621-7.
31. Evans G, Bryant HU, Magee D, *et al.* The effects of raloxifene on tibia histomorphometry in ovariectomized rats. *Endocrinology* 1994; 134:2283-8.
32. Ko BS, Kim DS, Kang S, *et al.* Wnt-signaling-mediated anti-osteoporotic activity of porcine placenta hydrolysates in ovariectomized rats. *Evid Based Complement Alternat Med* 2012; doi: 10.1155/2012/367698.
33. Cruz L, Assumpção E, Andradec SF, *et al.* Gastroresistant microparticles containing sodium alendronate prevent the bone loss in ovariectomized rats. *Eur J Pharm Sc*i 2010; 40:441-7.
34. Martins-Maciel ER, Campos LB, Salgueiro-Pagadigorria CL, *et al.* Raloxifene affects fatty acid oxidation in livers from ovariectomized rats by acting as a pro-oxidant agent. *Toxicol Lett* 2013; 217:82-9.
35. Vinerean HV. Rats–biology & husbandry. http://research.fiu.edu/facilities/acf/documents/rats-biology-husbandry.pdf. Accessed date: Aug 2014.
36. Hirayama M, Iijima S, Iwashita M, *et al.* Aging effects of major and trace elements in rat bones and their mutual correlations. *J Trace Elem Med Biol* 2011; 25:73-84.
37. Ikawa T, Kawaguchi A, Okabe T, *et al.* Hypergravity suppresses bone resorption in ovariectomized rats. *Adv Space Res* 2011; 47:1214-24.
38. Bertelloni S, Baroncelli GI, Mora S. Bone health in disorders of sex differentiation. *Sex Dev 2010*; 4:270-84.
39. Smith EP, Specker B, Korach KS. Recent experimental and clinical findings in the skeleton associated with loss of estrogen hormone or estrogen receptor activity. *J Steroid Biochem Mol Biol* 2010; 118: 264-72.
40. Yogesh HS, Chandrashekhar VM, Katti HR, *et al.* Anti-osteoporotic activity of aqueous-methanol extract of *Berberis aristata* in ovariectomized rats. *J Ethnopharmacol* 2011; 134:334-8.
41. Emons J, Chagin AS, Sävendahl L, *et al.* Mechanisms of growth plate maturation and epiphyseal fusion. *Horm Res Paedi*atr 2011; 75:383-91.
42. Lui JC, Baron J. Mechanisms limiting body growth in mammals. *Endocr Rev* 2011; 32:422-40.
43. Clemett D, Spencer CM. Raloxifene: a review of its use in postmenopausal osteoporosis. *Drugs* 2000; 60:379-411.
44. Hansdóttir H. Raloxifene for older women: a review of the literature. *Clin Interv Aging* 2008; 3:45-50.
45. Zirilli L, Maffei L, Meunier PJ, *et al.* The effects of long-term raloxifene and estradiol treatments on bone in a patient with congenital aromatase deficiency. *Bone* 2009; 45:827-32.
46. Shahnazari M, Yao W, Dai W, *et al.* Higher doses of bisphosphonates further improve bone mass, architecture, and strength but not the tissue material properties in aged rats. *Bone* 2010; 46: 1267-74.
47. Inoue Y, Shimojo N, Suzuki S, *et al.* Efficacy of intravenous alendronate for the treatment of glucocorticoid-induced osteoporosis in children with autoimmune diseases. *Clin Rheumatol* 2008; 27:909-12.
48. Bartl R, Frisch B, von Tresckow E, *et al.*, eds. Bisphosphonates in medical practice: actions-side effects-indications-strategies. Berlin: Springer, 2007.
49. Baron R, Ferrari S, Russell RGG. Denosumab and bisphosphonates: different mechanisms of action and effects. *Bone* 2011; 48:677-92.
50. Hsia J, Langer RD, Manson JE, *et al.* Conjugated equine estrogens and coronary heart disease: the Women's

Health Initiative. *Arch Intern Med* 2006; 166:357-65.
51. Armamento-Villareal R, Sheikh S, Nawaz A, *et al.* A new selective estrogen receptor modulator, CHF 4227.01, preserves bone mass and microarchitecture in ovariectomized rats. *J Bone Miner Res* 2005; 20: 2178-88.
52. Christodoulakos GE, Lambrinoudaki IV, Panoulis CP, *et al.* Effect of hormone replacement therapy, tibolone and raloxifene on serum lipids, apolipoprotein A1, apolipoprotein B and lipoprotein(a) in Greek postmenopausal women. *Gynecol Endocrinol* 2004; 18:244-57.
53. Schlegel W, Petersdorf LI, Junker R, *et al.* The effects of six months of treatment with a low-dose of conjugated oestrogens in menopausal women. *Clin Endocrinol* 1999; 51:643-51.
54. Darling GM, Johns JA, McCloud PI, *et al.* Estrogen and progestin compared with simvastatin for hypercholesterolemia in postmenopausal women. *N Engl J Med* 1997; 337:595-601.
55. Nanetti L, Camilletti A, Francucci CM, *et al.* Role of raloxifene on platelet metabolism and plasma lipids. *Eur J Clin Invest* 2008; 38:117-25.
56. Glueck CJ, Fallat RW, Scheel D. Effects of estrogenic compounds on triglyceride kinetics. *Metabolism* 1975; 24:537-45.
57. Kim HJ, Kalkhoff RK. Sex steroid influence on triglyceride metabolism. *Clin Invest* 1975; 56:888-96.
58. Sutherland MK, Brady H, Gayo-Fung LM, *et al.* Effects of SP500263, a novel selective estrogen receptor modulator, on bone, uterus, and serum cholesterol in the ovariectomized rat. *Calcif Tissue Int* 2003; 72:710-6.
59. Seidlová-Wuttke D, Christoffel J, Rimoldi G, *et al.* Comparison of effects of estradiol with those of octylmethoxycinnamate and 4-methylbenzylidene camphor on fat tissue, lipids and pituitary hormones. *Toxicol Appl Pharmacol* 2006; 214: 1-7.
60. Walsh BW, Schiff I, Rosner B, *et al.* Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991; 325:1196-204.
61. Lobo RA. Clinical review 27: effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. *J Clin Endocrinol Metab* 1991; 73:925-30.