Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Effect of atorvastatin on bone formation in ovariectomized rats

S.S. Salih and E.M. Al-Khashab

Department of Physiology, Biochemistry, and Pharmacology, Collage of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received May 08, 2022
Accepted October 13, 2022
Available online December 24, 2022

Keywords:
ALP
Atorvastatin
Ovariectomized rats
1,25 (OH)2D3

Correspondence:
E.M. Al-Khashab
elhelham91@gmail.com

Abstract

The study was designed in order the effect of one class of lipophilic statins (Atorvastatin) in some biomarkers of bone formation (Alkaline phosphatase (ALP), 1,25 $(OH)_2$ D3, calcium, and phosphorous) in serum of ovariectomized female rats. Thirty adult female rats (2.5-3) months, weighing (200-220) gm were housed at conditions of controlled temperature (22-25°C), cycle (12h light - 12h dark) in the house of animals of Veterinary Medicine College of Mosul University. The animals were divided into three equal groups, sham group, ovariectomized (ovx) group, and ovx treatment orally with 20mg/kg/d of atorvastatin. After 60 days of treatment, blood from all groups was collected for ALP activity, 1,25 $(OH)_2$ D3, calcium, and phosphorous estimation, and left femur bones were excised for histological examination. The results showed that the serum ALP, calcium, and phosphorus were significantly elevated, and a significant reduction in 1,25 $(OH)_2$D3 was noticed in the ovx group. However, treatment with atorvastatin caused a significant reduction in ALP with a non-significant elevation in 1,25 $(OH)_2$D3. Histological results showed a low density and thin trabecular bone, a few blood vessels, high numbers of osteoclast, with low numbers of osteoblasts in the ovx group. However, the treated ovx with atorvastatin increases the thickness of trabecular bone, medium developed osteogenic tissue, and a low number of osteoblasts. In conclusion, atorvastatin has a moderate effect on bone of ovx, affecting bone formation more than bone resorption.

DOI: 10.33899/ijvs.2022.133734.2287,

Introduction

Osteoporosis is a skeletal disease that affects millions of people in the world, and this disease accelerates bone loss leading to an increase in the probability of bone fracture. Menopause stimulates the loss of bone, and ovariectomy leads to bone loss because of estrogen deficiency which causes osteoporosis in humans and rats (1-3). Statins are 3-hydroxy 3-methylglutaryl-coenzyme A reductase inhibitors used to lower cholesterol levels. These drugs act by inhibiting cholesterol biosynthesis in the liver; therefore, statins reduce the risk of atherosclerosis and heart diseases (4,5). Some studies have suggested that statins may positively affect bone metabolism. Thus, taking statins to lower lipids may also impact bone metabolism; thus, statins decrease lipids and improve disorders of bone (6). Besides lowering the level of cholesterol, statins can affect bone by the decrease other molecules that produce from the mevalonate pathway, such as isoprenoid precursors, farnesyl diphosphate, and geranyl geranyl diphosphate, thus inhibiting the function of osteoclasts (4). There are two types of statins lipophilic and hydrophilic, depending on their potency of dissolving in lipid-containing media or water. Lipophilic statins (simvastatin, fluvastatin, pitavastatin, lovastatin, and atorvastatin) can move in cells quickly; however, statins dissolve in water as (rosuvastatin and pravastatin) are hepatoselectivity (7,8). Atorvastatin (Lipodar®) is an agent that inhibits cholesterol synthesis by increasing LDL receptors on the surface of the hepatocytes. It is absorbed quickly after taking it (9).

The purpose of the current work was to explore the influence of atorvastatin (dissolve in lipids) in some biomarkers of bone on ovariectomized (ovx) female rats which are considered a model of osteoporotic postmenopausal women. Ovariectomized rats have been used as a model to study the loss of bone in osteoporotic postmenopausal women. This model of rats mimics the impairment in the tissue of bone in the spine and hip.

Materials and methods

Ethical approve

Scientific ethical committee on animal experimentation at College of Veterinary Medicine, University of Mosul, UM.VET.2021.053

Laboratory animals

Thirty adult female rats 2.5-3 months, weighing 200-220 gm were obtained and housed in an environment 22-25ºC, at 12-12 h dark-light in the house of animals of the College Veterinary Medicine. Water and a standard diet were provided to all animals.

Experimental design

Thirty rats were equally grouped into the following: (10 rats/ group) and divided into the following: Group 1: Sham ovaries were not removed, sham-operated, and left about 30 days after surgery) furthermore, given orally distilled water for 60 days. Group 2: ovx (ovaries were removed and left 30 days after ovariectomy) served as a model of postmenopausal osteoporosis and given orally distilled water for 60 days. Group 3: (ovaries were removed and left 30 days after ovariectomy, then treated orally with 20mg/kg (9) of atorvastatin (lipodar/dar aldawa/Jordan) for 60 days.

Ovariectomy

After anesthesia with a mixture of (5 mg/kg) of xylazine hydrochloride and 50 mg/kg of a 10% ketamine given intraperitoneal (10,11), the abdominal region with skin and musculature was incised under the last rib, then ovary was excised. The sham group did the same procedure, but the ovary was not removed.

Blood collection

After 60 days of the treatment, by using the capillary tubes, samples of blood were collected from the retro-orbital plexus (12,13) from all groups, then drawn in a gel containing tubes, and leave it to clot after that put in the centrifuge for 15 min at 3000 rpm, serum separated and kept in -18°C for biomarkers examination.

Biomarkers of bone

Biomarkers were measured included: Rat alkaline phosphatase (ALP) activity, which was estimated by using the ELISA kit (catalog No: E-EL-R1109) from Elabscience company which has been used for research only. This ELISA kit uses the sandwich-ELISA principle that the micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to rat ALP, and the optical density is measured spectrophotometrically at a wavelength of 450±2nm. $1,25(OH)_2D3$ was estimated by using the ELISA kit (catalog No: E-EL-0016) from Elabscience company. Serum calcium was measured via spectrophotometry using diagnostic reagent kits (Biolabo, France), which include reacting calcium with the O-Cresol Phthalein to produce a complex color absorbed at 570 nm (14,15). Phosphorus in serum was determined by using Architect system operations which depend on inorganic phosphate reaction with ammonium molybdate to produce complex absorbance proportional directly to the level of inorganic phosphate in the serum.

Histological examination

In order to determine the osteoblast's and osteoclast's activity, left femur bones were sliced, then fixed in a solution of formalin 10% for 24h. After that, they were decalcified in formic acid 10% and embedded in paraffin blocks. Hematoxylin and eosin were used for staining the paraffin blocks; histological sections were photographed with Omax digital camera type (16).

Statistical analysis

Mean ± SE was used to describe the results, evaluated using sigma plot version 12.5. The one-way analysis of variance (ANOVA) test was used to compare all groups with a probability of $P \leq 0.05$.

Results

A significant elevation was noticed in ALP activity in the ovx group compared to the group, as shown in table 1, but the treatment with 20mg/kg/d of atorvastatin caused a significantly decreased ALP in comparison to the ovx group but did not reach the sham group level. Ovariectomy results in a significant decrease in $1,25(OH)_2D3$ level compared with the sham group, while treatment with 20mg/kg/d of atorvastatin caused a non-significant increase in $1,25(OH)_2D3$ compared with the ovx group. Levels of calcium and phosphorus were significantly increased in the ovx group compared to the sham group, but the treatment with 20mg/kg/d of atorvastatin caused a non-significantly decrease in the calcium and phosphorus levels in comparison to the ovx group.

The histological results of bone femur rats showed intact cartilage, high density and thickness of trabecular bone, and well-developed osteogenic tissue with few osteoclasts in the sham group, as shown in figure 1. Ovariectomy caused a low density and thin trabecular bone. In addition, there were a few blood vessels and poorly developed osteogenic tissue. There are high numbers of osteoclasts with low numbers of

osteoblasts, as seen in figure 2. This means there was a change in bone caused by estrogen deficiency that leads to a condition similar to the change in the bone seen in women with postmenopausal osteoporosis. The atorvastatin-treated group showed a high-density trabecular bone (TB), few blood vessels (BV), and medium-developed osteogenic tissue (OT) (Figure 3). A significant reduction was shown in the thickness of trabecular bone/µm of ovx compared to the sham group shown in table 2, the group treated with atorvastatin 20mg/kg/d led to a significant elevation in thickness of trabecular bone in comparison to ovx group. These results mean an improvement in the bone of the ovx rat after the treatment with atorvastatin.

Table 1: Effect of atorvastatin on serum ALP, Vit D3, Ca, and P levels in ovx rats

Biomarkers	Sham	OVX	OVX + A
ALP (ng/ml)	11.64 ± 0.56 [c]	16.79 ± 0.34 [a]	14.18 ± 0.66 [b]
1,25(OH)$_2$D3 (pg/ml)	223.70 ± 4.52 [a]	164 ± 4.62 [b]	177 ± 5.39 [b]
Ca (mg/dl)	10.90 ± 0.08 [b]	11.51 ± 0.11 [a]	11.28 ± 0.10 [a]
P (mg/dl)	4.15 ± 0.42 [b]	5.74 ± 0.13 [a]	5.30 ± 0.27 [a]

Mean ± SE with various superscript letters in the identical row mean significant at P≤0.05. n=10 rats/ group.

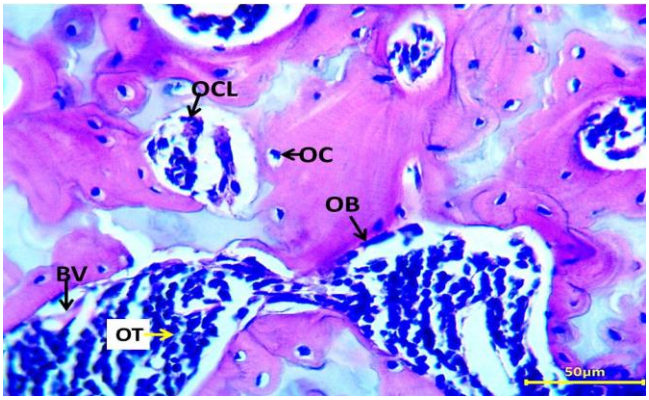

Figure 1: The photomicrograph of rat femur bone of (the Sham) group shows normal architecture represented by osteoblasts (OB), osteocytes (OC), few osteoclasts (OCL), and blood vessels (BV). H&E stain, Scale bar=50µm, 400X.

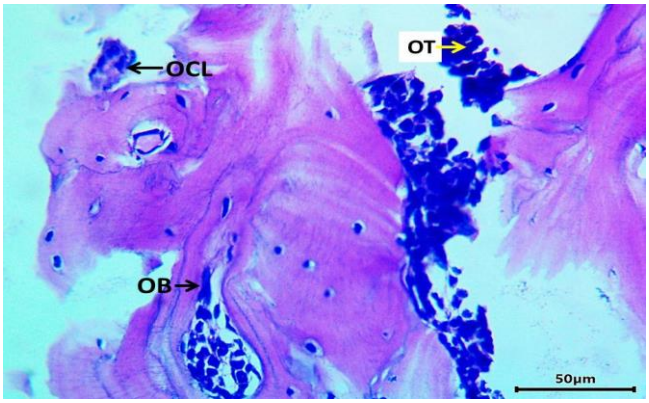

Figure 2: The photomicrograph of rat femur bone of (OVX) group shows the presence of a low number of osteoblasts (OB), high numbers of osteoclasts (OCL), and poor developed osteogenic tissue (OT). H&E stain, Scale bar=50µm, 400X.

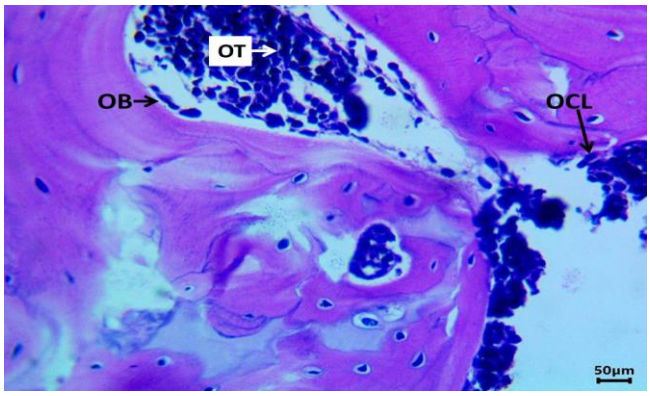

Figure 3: The photomicrograph of rat femur bone of atorvastatin treated group shows the presence of medium numbers osteoblasts (OB), medium numbers osteoclasts (OCL), and medium developed osteogenic tissue (OT). H&E stain, Scale bar=50µm, 400X.

Table 2: Thickness of trabecular bone of all groups

Groups	Thickness of trabecular bone /µm
sham	86.6 ± 12.4 [a]
Ovx	50 ± 4.2 [b]
OVX + A	69.6 ± 8.2 [a]

Mean ± SE with various superscript letters in the identical row mean significant P≤0.05. n=10 rats/group.

Discussion

Our study aimed to investigate the influence of the atorvastatin (lipophilic statin) in the bone formation of osteoporotic female rats caused by ovariectomy. Ovariectomy results in estrogen deficiency which leads to changes in bone. Ovariectomized rats were used as a model to study postmenopausal bone loss because the mimic deterioration in the rats bone is similar to estrogen reduction

seen in women with osteoporosis. So, the change in bone in rats is similar to that seen in estrogen-deficient osteoporotic women (17,18).

In our study, the level of ALP activity was elevated significantly in the ovx group compared to the sham group. This result agrees with Morgan *et al.* (19) and Grigoryan *et al.* (20) they noticed that the activity of ALP was significantly elevated in ovx rats. Also, our results agree with our prior study, which showed an increase in the level of ALP activity in the ovx group compared to the sham group (21). The decrease in estrogen production by ovaries is related to high bone turnover and high levels of markers related to bone formation and resorption. Ovariectomy increases ALP. This elevation is due to the unbalance in bone remodeling and deficiency of estrogen. This elevation in ALP refers to a high turnover of ovx rats characterized by an elevation in both formation and resorption, but the resorption process is more than the formation process. Estrogen inhibiting osteoclastogenesis, osteoclastogenesis process, and bone remodeling involve signaling pathways, like osteoprotegerin, RANK ligand, and RANK. RANK ligand stimulates activation and osteoclast differentiation and inhibits apoptosis in this cell type (22,23).

In our study, treatment of the ovx group with 20mg/kg of atorvastatin led to a significant reduction in ALP activity compared to the ovx group, meaning there is a significant improvement in the formation of bone and a decrease in bone resorption. Serum ALP is one of the biomarkers formations of bone. ALP levels, especially ALP specific for bone, are elevated in osteoporosis and in some metabolic bone diseases (24). This result indicates the utility of using atorvastatin in bone formation, and atorvastatin efficiently ameliorated ovx-induced osteoporosis. This study's increase in ALP activity agrees with the result of Sabry *et al.* (25). They noticed an elevation in the serum ALP in the ovx group. In our study, the reduction in ALP in ovx rats treated with 20mg/kg atorvastatin agreed with the results of El-Nabarawi *et al.* (26) they showed a reduction in serum ALP after treatment with atorvastatin.

ALP plays an essential role in the formation of hard tissue. It increases and facilitates mineralization. Also, it decreases pyrophosphate levels in the fluids out of the cell. It has a crucial role in the mechanism of calcification. ALP is produced through growth and is local on the surface of the cell and matrix vesicles in all tissues (27). Our study showed a significant reduction in serum $1,25(OH)_2D3$ in the ovx group compared with the sham group. Ovariectomy leads to an increase in the risk of osteoporosis, and this resembles postmenopausal osteoporotic women. In addition, ovariectomized rats are an excellent standard model for examining the drugs used for the treatment and prevention of osteoporosis. Ovariectomy results in estrogen deficiency that is known to decrease $1,25(OH)_2D3$ (28). Our results agree with Muhammad *et al.* (29) they noticed a decrease in serum 25(OH)D in the ovx group. Vitamin D is considered a pro-hormone, which is necessary for calcium homeostasis. $1,25(OH)_2D3$ stimulates the absorption of calcium and phosphorus from the intestine and improves the mineralization of the skeleton. In addition, $1,25(OH)_2D3$ with parathyroid hormone stimulates the mobilization of calcium from bone in order to prevent hypocalcemia (30,31).

Some researchers have shown a valuable role of statins on levels of $1,25(OH)_2D3$, but other studies have had no effect. Our results showed that rosuvastatin and atorvastatin could not reveal a significant influence on serum 25(OH)D level (32). However, in a study on patients with hyperlipidemia, they were given 10mg of rosuvastatin led to a significant increase in 25(OH)D levels (33). The exact mechanism of statins effect on 25(OH)D metabolism is not understood, but some researchers have proposed that 7-dehydrocholesterol is the precursor of vitamin D and cholesterol, and the inhibition of 3-hydroxy-3methylglutaryl coenzyme A reductase leads to an elevation in 7-dehydrocholesterol levels, this may offer a substrate for 25-hydroxy vitamin D synthesis (34).

Our presented study indicated calcium and phosphorus concentrations were significantly elevated in the ovx group compared to the sham group. This result agrees with Yousefzadeh *et al.* (35) they reported elevated serum phosphorus in ovariectomized rats from the first week and continued elevation until the ninth week. Also, Ca and P elevation in our study agrees with the study of (14) that showed a significant increase in Ca and P levels after ovariectomy. Our results indicated that bone turnover because of: estrogen deficiency causes a reduction in $[1,25(OH)_2D3]$ and a decrease in calcium absorption in the intestine. Reduction in serum calcium leads to parathyroid hormone PTH secretion that causes releasing of Ca and P from the skeleton to normalize serum Ca and P levels. This process leads to excretion of Ca and P in the urine and bone loss. Calcium levels in serum depend on the level of estrogen deficiency (28).

Also, our study's elevation in serum Ca agrees with Ohlsson *et al.* (36) and Naki (37). However, treatment of the ovx groups with 20mg/kg atorvastatin resulted in a non-significant reduction in Ca and P compared with ovx, which means this dose could not be returned Ca and P to the levels of the sham group. Also, the treatment with atorvastatin caused a non-significant decrease in P compared with the ovx group; this agrees with Romero *et al.* (24) they noticed a high level of P in all groups of their study. Calcium and phosphorus have an essential role in many functions of the body. Therefore, their regulations in plasma are made by the action of resorption/excretion in the kidney with absorption by the intestine and exchange from bone, which is considered the reservoir for Ca and P. The treatment of ovx groups with 20mg/kg atorvastatin leads to a non-significant decrease in serum Ca compared with the ovx group. Our results agree with Gokdemir *et al.* (9) that they reported increased calcium in the group treated with rosuvastatin. In

another study (38), they noticed a hypercalcemia condition in patients using atorvastatin, and they suggested that atorvastatin caused a high level of calcium in serum. In addition, serum calcium elevated significantly in rats treated with atorvastatin (39).

The histological results showed a reduction in trabecular bone thickness and a low number of osteoblasts, in addition to high numbers of osteoclasts in the ovx group. These results agreed with the results of Zhang *et al.* (40) and Burtis *et al.* (14) they noticed a significant 60% decrease in the volume of trabecular bone after ovariectomy. Also, there was increased osteoclastogenesis. The reduction of 50% in mass of trabecular bone, and the connectivity of trabecular bone results from osteoporosis. This means ovariectomy leads to bone loss. Atorvastatin results are a medium developed of osteogenic tissue and increase the thickness of trabecular bone (41).

Conclusions

In conclusion, the results denote that atorvastatin affects bone formation in ovariectomized rat. However, it has negative effect on serum calcium and phosphorus level, so care must be taken into consideration when menopausal women are treated with it through measurement levels of calcium and phosphorus. The limitation of our work was that we could not estimate levels of PTH and estrogens because of the high cost of kits.

Acknowledgment

The authors appreciate the people at the University of Mosul Veterinary College and the animal home for providing facilities that enabled us to complete this study.

Conflict of interests

There are no conflicts of interest declared by the authors.

References

1. Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM. A comprehensive overview on osteoporosis and its risk factors. Ther Clin Risk Manag. 2018;14:2029 -2049. DOI: 10.2147/tcrm.s138000
2. Gallagher JC, Riggs BL, DeLuca HF. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. J Clin Endocrinol Metab. 1980;51(6):1359-64. DOI: 10.1210/jcem-51-6-1359
3. Ris te li J, Winter WE, Kleerekoper M, Ris te li L. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th ed. USA Elsevier; 2015. 741-768 p.
4. Chamani S, Liberale L, Mobasheri L, Montecucco F, Al-Rasadi K, Jamialahmadi T, Sahebkar A. The role of statins in the differentiation and function of bone cells. Eur J Clin Invest. 2021;51(7):13534. DOI: 10.1111/eci.13534
5. Pinal I, Casal M, Mammen AL. Statins. Med Clin. 2018;150(10):398-402. DOI: 10.1016/j.medcli.2017.11.030
6. Xu H, Yang YJ, Yang T, Qian HY. Statins and stem cell modulation. Ageing Res Rev. 2013;12(1):1-7. DOI: 10.1016/j.arr.2012.03.006
7. Maritz FJ, Conradie MM, Hulley PA, Gopal R, Hough S. Effect of statins on bone mineral density and bone histomorphometry in rodents. Arterioscler Thromb Vasc Biol. 2001;21(10):1636 -1641. DOI: 10.1161/hq1001.097781
8. Climent E, Benaiges D, Pedro-Botet J. Hydrophilic or lipophilic statins. Front Cardiovasc Med. 2021;8:687585. DOI: 10.3389/fcvm.2021.687585
9. Gokdemir GS, Gokdemir MT, Kayhan H, Yokus B, Tasdemir E, Gul C, Baylan M. The effects of rosuvastatin and pravastatin on bone metabolism in diabetic rats. Ann Med Res. 2021;28(10):1933 -1938. DOI: 10.5455/annalsmedres.2020.11.1108
10. Mustafa HH, Ali AK, Cheng C, Radzi R, Fong L, Mustapha N, Dyary HO. Development of experimentally-induced periodontitis in a Sprague Dawley rat model. Iraqi J Vet Sci. 2021;35(4):765-769. DOI: 10.33899/ijvs.2021.128422.1573
11. Struck MB, Andrutis KA, Ramirez HE, Battles AH. Effect of a short-term fast on ketamine-xylazine anesthesia in rats. J Am Assoc Lab Anim Sci. 2011;50(3):344 -348. [available at]
12. Mohammed IA, Shaban KA, Albadrany YM. Hepato-renal and hematological effects of flunixin and silymarin coadministration in rats. Iraqi J Vet Sci. 2022;36(2):367-373. DOI: 10.33899/ijvs.2021.130323.1800
13. Al-Ameen SA, Jirjees EH, Tawfeeq FK. Effect of sodium benzoate on some biochemical, physiological and histopathological aspects in adult male rats. Iraqi J Vet Sci. 2022;36(2):267-272. DOI: 10.33899/ijvs.2021.129935.1705 .
14. Burtis CA, Ashwood ER. Tietz Textbook of clinical chemistry. 3rd ed. Philadelphia: WB Saunders Company; 1999. 1435-1439 p.
15. Al-Mamari AH. Effect of strontium chloride in some biochemical parameters in normal and ovariectomized rats [master's thesis]. Mosul: University of Mosul, Iraq; 2010.
16. Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. 34th ed. New York: McGraw-Hill Book Company; 1968. 67-70 p.
17. Johnston BD, Ward WE. The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women. Biomed Res Int. 2015;(6)35-23. DOI: 10.1155/2015/635023
18. Torrubia B, Martín-Fernández M, Rubert M, Gómez-Chinchón M, Sosa M. Effects of calcium and vitamin-d supplementation on bone quality in an ovariectomized rat model. J Surg. 2020;5:1276. DOI: 10.29011/2575-9760.001276
19. Morgan EN, Alsharidah AS, Mousa AM, Edrees HM. Irisin has a protective role against osteoporosis in ovariectomized rats. Biomed Res Int. 2021;2021:5570229. DOI: 10.1155/2021/5570229
20. Grigoryan AV, Dimitrova AA, Kostov KG, Russeva AL, Atanasova MA, Blagev AB, Betova TM, Trifonov RG. Changes of serum concentrations of alkaline phosphatase and metalloproteinase-9 in an ovariectomized wister rat model of osteoporosis. J Biomed Clin Res. 2017;10(1):32-36. DOI: 10.1515/jbcr-2017-0006
21. Hamdoon AA, Al-khashab EM, Al-Hashemi HM. Effect of saponin extract of glycyrrihiza glabra in activity of hepatic enzymes and some biochemical parameters in serum of adults ovariectomized female rats. Iraqi J Vet Sci. 2020;34(2):411-415. DOI: 10.33899/ijvs.2019.126127.1239
22. Alrowaili MG, Hussein AM, Eid EA, Serria MS, Abdellatif H, Sakr HF. effect of intermittent fasting on glucose homeostasis and bone remodeling in glucocorticoid-induced osteoporosis rat model. J Bone Metabol. 2021;28(4):307-316. DOI: 10.11005/jbm.2021.28.4.307
23. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M. Endochondral ossification: How cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol. 2008;40(1):46 -62. DOI: 10.1016/j.biocel.2007.06.009
24. Romero CM, Manrique AS, Rodríguez PM. Biochemical markers in osteoporosis: Usefulness in clinical practice. Reumatol Clin. 2012;8(3):149-152. DOI: 10.1016/j.reuma.2011.05.010
25. Sabry M, Mostafa S, Kamar S, Rashed L, Estaphan S. The cross-talk between matrix metalloproteinase-9, RANKL/OPG system and

cardiovascular risk factors in an ovariectomized rat model of postmenopausal osteoporosis. PLoS One. 2021;16(10):254-258. DOI: 10.1371/journal.pone.0258254
26. El-Nabarawi N, El-Wakd M, Salem M. Atorvastatin, a double weapon in osteoporosis treatment: An experimental and clinical study. Drug Des Devel Ther. 2017;11:1383 -1391. DOI: 10.2147/DDDT.S133020
27. Vimalraj S. Alkaline phosphatase: Structure, expression and its function in bone mineralization. Gene. 2020;754(14):48-55. DOI: 10.1016/j.gene.2020.144855
28. Nagareddy PR, Lakshmana M. Withania somnifera improves bone calcification in calcium-deficient ovariectomized rats. J Pharm Pharmacol. 2006;58(4):513-519. DOI: 10.1211/jpp.58.4.0011
29. Muhammad MH, Hussien NI, Elwia SK. Vitamin D replacement mitigates menopause-associated dyslipidaemia and atherogenic indices in ovariectomized rats: A biochemical study. Exp Clin Endocrinol Diabetes. 2020;128(3):144-151. DOI: 10.1055/a-0934-5666
30. Duchow EG, Duchow MW, Plum LA, DeLuca HF. Vitamin D binding protein greatly improves bioactivity but is not essential for orally administered vitamin D. Physiol Rep. 2021;9(23):15138. DOI: 10.14814/phy2.15138
31. Mustafa RA, Alfky NA, Hijazi HH, Header EA, Azzeh FS. Biological effect of calcium and vitamin D dietary supplements against osteoporosis in ovariectomized rats. Prog Nutr. 2018;20(1):86-93. DOI: 10.23751/pn.v20i1.5223
32. Anagnostis P, Adamidou F, Slavakis A, Polyzos SA, Selalmatzidou D, Panagiotou A, Athyros VG, Karagiannis A, Kita M. Comparative effect of atorvastatin and rosuvastatin on 25-hydroxy-vitamin d levels in non-diabetic patients with dyslipidaemia: A prospective randomized open-label pilot study. Open Cardiovasc Med. 2014;8:55-60. DOI: 10.2174/1874192401408010055
33. Sathyapalan T, Shepherd J, Atkin SL, Kilpatrick ES. The effect of atorvastatin and simvastatin on vitamin D, oxidative stress and inflammatory marker concentrations in patients with type 2 diabetes: A crossover study. Diabetes Obes Metab. 2013;15(8):767 -769. DOI: 10.1111/dom.12074
34. Orces CH, Montalvan M, Tettamanti D. The effect of statins on serum vitamin d concentrations among older adults. Cureus. 2020;12(7):8950. DOI: 10.7759/cureus.8950
35. Yousefzadeh N, Kashfi K, Jeddi S, Ghasemi A. Ovariectomized rat model of osteoporosis:a practical guide. EXCLI J. 2020;19:89 -107. DOI: 10.17179/excli2019-1990
36. Ohlsson C, Engdahl C, Fak F, Andersson A, Windahl SH, Farman HH, Moverare-Skrtic S. Islander U, Sjogren K. Probiotics protect mice from ovariectomy-induced cortical bone loss. PLoS One. 2014;9(3):92368. DOI: 10.1371/journal.pone.0092368
37. Naik P. Essentials of biochemistry. 1st ed. New Delhi: Jaypee Brothers ;2012. 276-292 p.
38. Ipekçi SH, Baldane S, Sözen M, Kebapçılar L. Can atorvastatin calcium cause asymptomatic hypercalcemia?. Turk Kardiyol Dern Ars. 2014;42(7):662-666. DOI: 10.5543/tkda.2014.05995
39. Kawane T, Terashima S, Kurahashi I, Yanagawa T, Yoshida H, Horiuchi N. Atorvastatin enhances bone density in ovariectomized rats given 17beta-estradiol or human parathyroid hormone (1-34). Endocrine. 2004;24(2):121-129. DOI: 10.1385/endo:24:2:121
40. Zhang L, Feng X, McDonald JM. The role of calmodulin in the regulation of osteoclastogenesis. Endocrinol. 2003;144(10):4536 -4543. DOI: 10.1210/en.2003-0147
41. Lane NE, Yao W, Kinney JH, Modin G, Balooch M, Wronski TJ. Both hPTH(1-34) and bFGF increase trabecular bone mass in osteopenic rats, but they have different effects on trabecular bone architecture. J Bone Miner Res. 2003;18(12):2105-2115. DOI: 10.1359/jbmr.2003.18.12.2105

تأثير الاتورفاستاتين على بناء العظم في الجرذان المستأصلة المبايض

سمية سلطان صالح والهام محمد الخشاب

فرع الفسلجة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم تصميم هذه الدراسة للتحري عن تأثير الأتورفاستاتين أحد أصناف الستاتينات المحبة للدهون في بعض المؤشرات الحيوية الخاصة ببناء العظم، أنزيم الفوسفاتيز القاعدي، ٢٥,١ ثنائي هيدروكسي فيتامين د٣، الكالسيوم والفسفور في مصل دم إناث الجرذان المستأصلة المبايض. إذ تم استخدام ثلاثون من إناث الجرذان البالغة تراوحت أعمارها ٢,٥ - ٣ أشهر وأوزانها ٢٠٠ - ٢٢٠ غم، والتي تمت تربيتها تحت ظروف مسيطر عليها عند درجة حرارة ٢٢±٣ °م و ١٢ ساعة ضوء/ ١٢ ساعة ظلام في بيت الحيوانات التابع لكلية الطب البيطري، جامعة الموصل. قسمت الحيوانات الى ثلاثة مجاميع متساوية وهي مجموعة السيطرة، المجموعة المستأصلة المبايض، والمجموعة المستأصلة المبايض المعاملة فمويا ب ٢٠ ملغم / كغم من الأتورفاستاتين. تم جمع الدم بعد ٦٠ يوم من المعاملة لتقدير فعالية الفوسفاتيز القاعدي، ٢٥,١ ثنائي هيدروكسي فيتامين د٣، الكالسيوم والفسفور. كما تم نزع عظم الفخذ الأيسر للفحص النسجي. أشارت النتائج الى ارتفاع فعالية أنزيم الفوسفاتيز القاعدي، الكالسيوم والفسفور معنويا في مصل الدم مع انخفاض معنوي في ٢٥,١ ثنائي هيدروكسي فيتامين د٣ في الجرذان المستأصلة المبايض، في حين أدت المعاملة بالأتورفاستاتين الى انخفاض معنوي في فعالية أنزيم الفوسفاتيز القاعدي مع زيادة غير معنوية في مستوى ٢٥,١ ثنائي هيدروكسي فيتامين د٣. كما أشارت نتائج الدراسة النسجية الى انخفاض كثافة العظم وترققها، إضافة الى قلة الأوعية الدموية وأعداد كبيرة من الخلايا الهادمة للعظم وقلة بالخلايا البانية للعظم في المجموعة المستأصلة المبايض. في حين أدت معاملة الجرذان المستأصلة المبايض بالأتورفاستاتين الى زيادة سمك العظام التربيقية ونمو متوسط في الأنسجة العظمية مع قلة الخلايا البانية للعظم. نستنتج من الدراسة الحالية أن الأتورفاستاتين يمتلك تأثير متوسط في عظام الجرذان المستأصلة المبايض وان تأثيره في بناء العظم يكون أكبر من تأثيره في ارتشاف العظم.