Bone Mineral Density and the Relationship between Lipid Profile and Bone Mineral Density in the Rats Administered Juniperus Communis Linn.

[1]ERSIN USKUN*, [2]MEHMET AKDOGAN, [3]MUSTAFA YILDIZ AND [1]YONCA SONMEZ
[1]*Suleyman Demirel University*, *Faculty of Medicine*, *Department of Public Health*, *Turkey.*
[2]*Suleyman Demirel University*, *Faculty of Medicine*, *Department of Biochemistry*, *Turkey.*
[3]*Suleyman Demirel University*, *Faculty of Medicine*, *Department of Nuclear Medicine*, *Turkey.*
ersinuskun@gmail.com*

(Received on 10th September 2011, accepted in revised form 13th December 2011)

Summary: The aim of the study is to investigate the relationship of Juniperus Communis Lynn. with the bone mineral density in the rats fed with a high cholesterol (1%) diet. Thirty five Wistar albino rats weighed approximately 250-300 were used in this study. The rats are divided in five groups of seven each. Groups I and II were administered 0.5 ml of 0.5% Sodium Carboxy Methyl Cellulose (SCMC), while Groups II, IV and V administered 0.5 ml of juniperus communis linn dissolved in 25, 50, 100 mg/kg. Group I and Group II were fed with normal pellets while the other four groups were fed with pellets containing 1% cholesterol. Levels of lipid profile and High Density Lipoprotein Cholesterol (HDL-C) were defined in all the groups. Furthermore, bone mineral density (B M D) of the animals were obtained with DEXA scanner. BMD values of the rats did not show a different among the groups. Significant negative correlations were determined between BMD measurements and LDL-C levels in all groups connected with dose of Juniperus Communis oil. However, this relationship was not linear.

Keywords: Juniperus Communis Linn., Bone Mineral Density, Lipid and lipoproteins

Introduction

Osteoporosis that is known as low bone mass and deteriorating of the bone microarchitecture results in an increase of the bone fragility. Although its pathogenesis yet cannot be fully explained, recently it is emphasized that the bone mineral turnover and adipose tissue are in a close relationship [1]. Bone loss is in concurrence with an increase of the fatty tissue in the bone marrow, thus considering osteoblasts and adipocytes differentiate from the same progenitor cells in the bone marrow, the importance of this process could be better understood [2].

The cells playing a main role in the bone mechanism are osteoblasts and osteoclasts. Osteoclasts realize the resorption, while osteoblasts take an active role in the mineralization and remodeling process [3].

Oxidation of the lipoproteins inhibits the osteoblast differentiation, resulting in a decrease in the bone mineral density (BMD). It has been suggested that elevation at the oxide lipoprotein level implies a risk for cardiovascular disease, and the free radicals negatively affect the bone cycle. It is known that the synthesis of atherogenic acting lipids is inhibited during the osteoblastic differentiation and therefore, deceleration of the bone mineral cycle is associated with hypercholesterolemia and hyperlipidemia [4].

In the studies with statins, it has been shown that the statins induces the bone formation in vivo and in vitro [5] and there is a significant decreasing on the spontaneous fractures after statin administration associated with the bone density [6-12]. Statins increase the bone formation by increasing type I collagen, osteocalcina and bone morphogenetic protein II [3]. Lipid lowering medications to increase the bone formation and BMD increasing drugs to decrease the lipid levels of serum suggest an association between the osteoporosis and hyperlipidemia [4].

Today, numerous studies have been conducted about the effects of statins on the bone mineral density and risk of the fracture [9, 13-15]. On the other hand, the idea that use of the bone specific statins or carrier systems to carry a higher amount of statins to the bone to provide positive clinical outcome is investigating in the ongoing studies [16].

Recently, widely popular herbal medicine (phytotherapie) approaches become a current issue also in the treatment of the high levels of cholesterol. Phytos or products derived from the plants such as fennel, thymus, rosemary, artichoke leaf, dandelion, lemon juice and grape juice are used by the people for this purpose [17].

Juniperus species are known to be used as a panacea in the medieval age [18]. Fruit and oil of the juniperus which has aromatic features are used in the

*To whom all correspondence should be addressed.

field of phytotherapies and medicine for at least three centuries [19-21]. Juniperus Communis Linn tree with green leaves during four seasons is one of the juniperus species in the northern hemisphere. Over the time, juniperus has been used to relieve kidney and bladder disorders [20] and peristaltism (movements), as a carminative and for some disorders such as rheumatism and arthritis [19-21]. It has been demonstrated that juniperus fruits have antioxidant characteristics [22], decreased the blood glucose in the animal models [23, 24] and have antiherpetic [25], antimicrobial [26] and antifungal [27] effects. Besides these effects, isoprenoids are known from several studies to have hypolipidemic effects [28]. Statins which are used for treatment of hyperlipidemia in the modern medicine inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG co-A) reductase enzyme that is the rate limiting enzyme of the cholesterol synthesis, increasing number of the LDL receptor in the liver and provide intake of the LDL cholesterol from the plasm to the liver [29]. In in-vitro and in-vivo studies, it has been stated that limonene which is existed in the juniperus prevents LDL oxidation and thus can slow down the atherosclerotic progress [30]. Role of the hypercholesterolemia in the pathogenesis of coronary heart disease (CHD) has been clearly demonstrated and particularly decreasing of the serum cholesterol with HMG-CoA reductase enzymes has been shown in several clinical studies to decrease coronary problems.

Juniperus fruit and oil those are subject to this study have been used among the people for a long time to treat different diseases and numerous studies have been conducted about their effects. However, there is not any study which has investigated whether the extract of this fruit is associated with the bone mineral density as in the statins.

Considering deceleration in the bone mineral cycle is linked to hypercholesterolemia and hyperlipidemia and lipid lowering medications increase the bone formation in the literature, in this study, we aimed to investigate the relationship of the juniperus oil with the bone mineral density in the rats with hypercholesterolemia created.

Results and Discussion

In this study, giving juniperus oil at different amounts to the rats with hypercholesterolemia created, differences between the bone mineral densities of the rats and the relationship between lipid profile and bone mineral density were examined.

The weights of the rats at the beginning (pre) and ending (post) of the study are shown in Table-1. The pre and post weights of rats were found significantly different. The weights of rats were increased at the end of the study. The results of the BMD measurements and biochemical parameters (Total Cholesterol [TC], Triglyceride [TG], Low Density Lipoprotein Cholesterol [LDL-C] and High Density Lipoprotein Cholesterol [HDL-C]) are shown in Table-2. The P value of the comparisons between the groups are shown in Table-3. HDL-C was different between Group I and II. It was also different between group I and group V. Correlation coefficients between BMD and biochemical parameters are shown in Table-4. Significant negative correlations were determined between BMD measurements and LDL-C levels in group II, III, IV and V. Lineer regression coefficients between BMD and biochemical parameters in the groups are shown Table-5.

Table-1: The weights of the rats at the begining (pre) and ending (post) of the study.

	Group I	Group II	Group III	Group IV	Group V
Weight(g)-pre	237.1±27.3	212.9±2.3	235.0±22.9	222.1±2.8	224.7±11.1
Weight(g)-post	287.3±24.9	277.7±3.4	302.8±22.9	286.1±3.8	292.3±13.7
P Value	0.018	0.018	0.028	0.018	0.018

Group I: Control; Group II: Hipercolesterolemic; Group III: Hipercolestrolemic and treated with 25 mg/kg JCL Oil; Group IV: Hipercolestrolemic and treated with 50 mg/kg JCL Oil; Group V: Hipercolestrolemic and treated with 100 mg/kg JCL Oil.

Table-2: The results of the measurements in rats.

	Group I *Mean (SD)*	Group II *Mean (SD)*	Group III *Mean (SD)*	Group IV *Mean (SD)*	Group V *Mean (SD)*
BMD (g/cm^2)	0.113 (0.007)	0.115 (0.004)	0.117 (0.004)	0.109 (0.009)	0.117 (0.010)
TC (mg/dL)	61.9 (11.7)	52.3 (7.2)	59.2 (9.5)	59.7 (11.4)	55.4 (5.9)
TG (mg/dL)	44.0 (14.2)	37.7 (12.0)	40.0 (11.7)	51.4 (22.9)	48.1 (16.2)
LDL-C (mg/dL)	11.6 (7.0)	12.0 (2.9)	13.2 (1.9)	14.4 (4.4)	11.9 (3.3)
HDL-C (mg/dL)	41.4 (6.3)	32.6 (4.9)	37.8 (7.5)	35.0 (5.1)	33.7 (5.0)

Group I: Control; Group II: Hipercolesterolemic; Group III: Hipercolestrolemic and treated with 25 mg/kg Juniperus Oil; Group IV: Hipercolestrolemic and treated with 50 mg/kg Juniperus Oil; Group V: Hipercolestrolemic and treated with 100 mg/kg Juniperus Oil.

High levels of blood cholesterol are one of the main causes of the important health problems, especially atherosclerosis and heart diseases for people of today. Therefore, keeping the cholesterol levels of blood within normal ranges is crucial to prevent the progress of many diseases.

Table-3: The P values of the comparisons between the groups

Groups	BMD P value	TC P value	TG P value	LDL-C P value	HDL-C P value
Group I-Group II	0.383	0.097	0.259	0.456	0.011
Group I-Group III	0.101	0.836	0.731	0.366	0.534
Group I-Group IV	0.805	0.620	0.535	0.259	0.073
Group I-Group V	0.259	0.383	0.710	0.535	0.038
Group II-Group III	0.366	0.101	0.445	0.445	0.073
Group II-Group IV	0.318	0.209	0.456	0.209	0.620
Group II -Group V	0.456	0.383	0.165	1	0.456
Group III-Group IV	0.073	0.445	0.534	0.836	0.181
Group III-Group V	0.731	0.234	0.366	0.628	0.181
Group IV-Group V	0.165	0.383	0.710	0.318	0.383

Table-4: Correlation coeficients (rho) and P values between BMD and biochemical parameters in the groups

	BMD				
	Group I	Group II	Group III	Group IV	Group V
	Rho (P value)	Rho (P value)	Rho (P value)	Rho (P value)	Rho (P value)
TC (mg/dL)	-0.286 (0.535)	-0.108 (0.818)	0.000 (1.000)	-0.739 (0.058)	-0.214 (0.645)
TG (mg/dL)	0.072 (0.878)	0.037 (0.937)	0.371 (0.468)	-0.429 (0.337)	0.847 (0.016)
LDL-C (mg/dL)	-0.143 (0.760)	-0.342 (0.042)	-0.522 (0.028)	-0.844 (0.017)	-0.649 (0.015)
HDL-C (mg/dL)	-0.324 (0.478)	-0.436 (0.328)	-0.406 (0.425)	-0.396 (0.379)	-0.429 (0.337)

Developing safe and more effective drugs are in scope of the responsibility of pharmacology and pharmaceutical science fields. Today there are a lot of medicines using for the treatment of hypercholesterolemia. Lipid lowering agents Statins, which are HMG-CoA reductase inhibitors are commonly used to reduce blood lipid levels and to treat cardiovascular disease. Recent evidence statins have potent positive effects on bone formation in rodents [5], and statin therapy in humans correlates with reduced osteoporosis, and bone fractures [6-8].

In this study, Juniperus communis oil that is one of the herbal products using in the metabolism disorders and as a lipid regulator among the people was given at different amounts, and the changes were examined. Any other study which has investigated the relationship between juniperus communis oil and bone density was not encountered in the literature. In our study in which such a relationship was examined for the first time, juniperus communis oil extract that is used among the people to regulate the lipid level of blood was defined not to create any significant change in the bone mineral densities of the rats.

Table-5: Lineer regression analyses and coeficients (B) and P values between BMD and biochemical parameters in the groups.

	BMD				
	Group I	Group II	Group III	Group IV	Group V
	B (P value)	B (P value)	B (P value)	B (P value)	B (P value)
Constant	0.117 (0.214)	0.117 (0.002)	0.103 (0.043)	0.077 (0.157)	0.050 (0.349)
TC (mg/dL)	-0.017 (0.506)	0.011 (0.148)	-0.008 (0.230)	0.020 (0.211)	-0.044 (0.367)
TG (mg/dL)	0.003 (0.506)	-0.002 (0.147)	0.002 (0.197)	-0.004 (0.198)	0.010 (0.346)
LDL-C (mg/dL)	0.017 (0.522)	-0.009 (0.191)	0.009 (0.184)	-0.023 (0.196)	0.044 (0.385)
HDL-C (mg/dL)	0.018 (0.498)	-0.012 (0.137)	0.007 (0243)	-0.017 (0.229)	0.046 (0.354)
Adjusted R^2	-0.940 (0.875)	0.843 (0.102)	0.843 (0.263)	0.336 (0.393)	0.496 (0.308)

High lipid levels [4, 31-33], atherosclerosis [35], cardiovascular calcification (Barengolts *et al*., 1998), and cardiovascular disease mortality [36] are associated closely with Low bone mineral density. It is known that minimally oxidized low-density lipoprotein, and other bioactive oxidized lipids that promote atherogenesis and are increased in atherosclerotic lesions [37], also inhibit osteoblastic differentiation of bone- and marrow-derived preosteoblasts in vitro [38]. Consistent with the literature, in this study, a negative correlation was defined between LDL-C and BMD in the rats groups out of the controls those received special feed to create an experimental hypercholesterolemia. BMD decreased as the LDL-C increased. Although (HDL are recognized for their anti-atherogenic action, there is less information about their ability to protect against osteoporosis. Therefore, Brodeur and *et al*. [39] investigated the capacity of HDL to prevent the cell death induced by OxLDL in human osteoblastic cells. They found in conclusion that HDL may exert beneficial actions on bone metabolism. Whereas in this study, there was no any correlation found between HDL-C level and bone density.

The negative correlation between LDL-C and BMD that had been reported in the pervious studies [4, 32, 33] was also seen in all the rats in our study out of the controls. With another words, intake and dose of the juniperus oil was defined a negative correlation between LDL-C and BMD. The negative correlation existing between LDL-C and BMD in the rats with hypercholesterolemia created continues in a significant way in the rats with juniperus oil used at different doses. BMD decreases as LDL-C increases. However, any significant relationship between BMD and biochemical parameters in the groups were not determined in linear regression analyses. It was concluded from this evaluation that hyper-cholesterolemia decreases BMD, and dose of the

Juniperus Communis oil in hypercholesterolemia affect the relationship between LDL-C level and BMD. However, the relationship which was determined between BMD and LDL-C was not linear relationship.

Experimental

This study was carried out in Suleyman Demirel University, Faculty of Medicine, the Experimental Animal Laboratory, and the Dept: of Medical Biochemistry, Research Laboratory.

Experimental Animals

In this study, 35 adult male Wistar albino rats with weights differ between 200-250 g, obtained from Suleyman Demirel University, Experimental Animal Laboratory, were used. The animals were divided into 5 equal groups of 7: a control group (group I), a group receiving a diet supplemented with 1% cholesterol (group II), groups receiving a diet supplemented with 1% cholesterol and 25-50-100 mg/kg of Juniperus Communis Linn. (JCL) oil (group III: 25 mg/kg JCL, group IV: 50 mg/kg JCL, group V: 100 mg/kg JCL). The rats were kept under standard light (12 hours of day light/12 hours of darkness) and at a temperature of 25ºC. They were fed 30 days in specially prepared cages.

On the first day of the study, the body weight of the rats were measured. The experiment was ended with the collection of blood samples and bone tissues under ketamine anaesthesia at 30 days of the study.

Preparation of Samples

At the end of 30 days, all groups were decapitated under anaesthesia (Ketamine 90 mg/kg + Xylazine 10 mg/kg). Approximately 6-7 ml of blood samples from the abdominal aorta were collected into standard tubes, citrate tubes and EDTA tubes placed in ice. While the tubes without anticoagulant and the citrate tubes were centrifuged at 4000 rpm for 10 minutes at room temperature, the EDTA tubes were centrifuged in a cooling centrifuge. The serum from the tube without anitcoagulant was analyzed for levels of TC, TG, LDL-C and HDL-C on the same day using an Olympus AU2700 autoanalyser (Japan) and the TG Olympus brand commercial kit.

Measurement of the Bone Mineral Density

Tibial BMD (grams per g/cm^2) of the animals were obtained with DEXA scanner (Norland XR-46 bone densitometer, Norland Corp., Fort Atkinson, WI) using a small animal scan software (available from Norland). The scan resolution was 0.5 3 0.5 mm, and scan speed was 60 mm/sec. To minimize the interobserver variations the same technician carried out all analyses. Measuring one rat repeated three times assessed the reproducibility of the measurement system. The coefficient of variation (cv) was 1% for these measurements.

Prior to this study, the protocol was reviewed and approved by the Ethics Commitee for Animal Research.

Statistical Analysis

Data were analyzed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc, Chicago, IL, USA). Results were expressed as mean±SD. A P-value of $P<0.05$ was considered significant. Comparison between two independent groups was assessed by Mann Whitney U-test. Wilcoxon test was used for comparison of pre and post measurements. Correlations between BMD and biochemical parameters (TC, TG, LDL-C, HDL-C) were tested by the calculation of Spearman's rho correlation coefficients. Linear regression analyses were performed to explain the relationship between BMD and biochemical parameters (TC, TG, LDL-C, HDL-C).

Conclusion

In conclusion, consistent with the evidence exciting in the literature, a negative correlation was defined between hypercholesterolemia and BMD. Although use of Juniperus Communis oil was found to create the negative correlation between LDL and BMD connected with dose of Juniperus Communis oil, this relationship was not linear.

We believe that the results of this study will provide a new contribution to the studies about the use of juniperus oil that is one from the fashionable applications in recent years. Further controlled in-vivo and in-vitro studies on this subject with wider groups are needed.

References

1. R. E. Cole, *American Journal of Clinical Nutrition*, **108**, 289 (2008).
2. S. Adami, V. Braga, M. Zamboni, D. Gatti, M. Rossini, J. Bakri and E. Battaglia, *American Journal of Clinical Nutrition*, **74**,136 (2004).
3. X. Wang, H. Tokuda and D. Hatakeyama, *Archives of Biochemistry and Biophysics*, **415**, 6 (2003).
4. T. Majima, A. Shimatsu, Y. Komatsu, N. Satoh, A. Fukao, K. Ninomiya, T. Matsumura and K. Nakao, *Endocrine Journal*, **55**, 143 (2008).

5. G. Mundy, R. Garrett, S. Harris, J. Chan, D. Chen, G. Rossini, B. Boyce, M. Zhao and G. Gutierrez, *Science*, **286**, 1946 (1999).
6. C. R. Meier, R. G. Schlienger, M. E. Kraenzlin, B. Schlegel and H. Jick, *The Journal of the American Medical Association (JAMA)*, **283**, 3205 (2000).
7. P. S. Wang, D. H. Solomon, H. Mogun and J. Avorn, *The Journal of the American Medical Association (JAMA)*, **283**, 3211 (2000).
8. K. A. Chan, S. E. Andrade, M. Boles, D. S. M. Buist, G. A. Chase, J. G. Donahue, M. J. Goodman, J. H. Gurwitz, A. Z. LaCroix and R. Platt, *Lancet*, **355**, 2185 (2000).
9. J. A. Pasco JA, M. A. Kotowicz MA, M. J. Henry, K. M. Sanders and G. C. Nicholson, *Archives of Internal Medicine*, **162**, 537 (2002).
10. P. Andrea, B. Fiorenza, C. Bruno, R. Monica, P. Rodolfo and C. P. Giorgio, *Obstetrics & Gynecology*, **102**, 922 (2003).
11. M. W. Schoofs, M. C. Sturkeboom, M. van der Klift, A. Hofman, H. A. Pols and B. H. Stricker, *Journal of Bone and Mineral Research*, **19:** 1525 (2004).
12. D. H. Solomon, J. Avorn, C. F. Canning and P. S. Wang, *American Journal of Medicine*, **118**, 1414 (2005).
13. Q. O. Tang, G. T. Tran, Z. Gamie, S. Graham, E. Tsialogiannis, E. Tsiridis, T. Linder and E. Tsiridis, *Expert Opinion Investigational Drugs*, **17**, 1435 (2008).
14. T. Maeda, A. Matsunuma, T. Kawane and N. Horiuchi, *Biochemical Biophysicsical Research Communication*, **280**, 874 (2001).
15. J. Osuga, *Clinical Calcium*, **14**, 235 (2004).
16. F. Pepedil, G. S. Güven, *Medical Journal of Hacettepe*, **40**, 169 (2009).
17. T. Baytop, *Therapy with medicinal plants in Turkey,* 2nd ed, Nobel Tıp Press: Istanbul (1999).
18. E. Gurkan, *Herbal Treatment*, Marmara University Press: Istanbul (2003).
19. ESCOP, *"Juniperi fructus." Monographs on the Medicinal Uses of Plant Drug*, European Scientific Cooperative on Phytotherapy: Exeter, U.K (1997).
20. C. Newall, L. A. Anderson and J. D. Phillipson, *Herbal Medicines: A Guide for Health-Care Professionals*, Pharmaceutical Press: London, England (1996).
21. A. Y. Leung and S. Foster, *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. (2nd ed), John Wiley & Sons, Inc.: New York (1996).
22. M. Burits, K. Asres and F. Bucar, *Phytotherapy Research*, **15**, 103 (2001).
23. F. Sanchez de Medina, M. J. Gamez, I. Jimenez, J. Jimenez, J. I. Osuna and A. Zarzuelo, *Planta Medica*, **60**, 197 (1994).
24. R. Petlevski, M. Hadzija, M. Slijepcevic and D. Juretic, *Journals of Ethnopharmacol*, **75**, 181 (2001).
25. T. Markkanen, *Drugs Exp of Clinical Reearchs*, **7**, 691 (1981).
26. W. H. Johnston, J. J. Karchesy, G. H. Constantine and A. M. Craig, *Phytotherapy Research*, **15**, 586 (2001).
27. N. Filipowicz, M. Kaminski, J. Kurlenda, M. Asztemborska and J. R. Ochocka, *Phytotherapy Research*, **17**, 227 (2003).
28. V. N. Syrov, M. K. Abzalova, Z. A. Khushbaktova and M. B. Sultanov, *Pharmaceutical Chemistry Journal* **19**, 270 (1985).
29. P. A. Edwards and J. Ericsson *Courrent Opinion of Lipidolgy*, **9**, 433 (1998).
30. J. L. Witzum and D. Steinberg, *Journal of Clinical Invesigational Research*, **88**, 1785 (1991).
31. F. Parhami, Y. Tintut, W. G. Beamer, N. Gharavi, W. Goodman and L. L. Demer,, *Journalof Bone Mineral Research*, **16**, 182 (2001).
32. P. Orozco and I. Hurtado, *Osteoporosis International*, **11**, 103 (2000).
33. F. Parhami, A. Garfinkel and L. L. Demer, *Arterioscler Thrombosis and Vascular Biology*, **20**, 2346 (2000).
34. M. Laroche, J. M. Pouilles, C. Ribot, P. Bendayan, J. Bernard, H. Boccalon and B. Mazieres, *Clinical Rheumatoogyl*, **13**, 61 (1994).
35. E. I. Barengolts, M. Berman, S. C. Kukreja, T. Kouznetsova, C. Lin and E. V. Chomka, *Calcierous Tissue Integrity*, **62**, 209 (1998).
36. P. von der Recke, M. A. Hansen and C. Hassager *American Journal of Medicine*, **106**, 273 (1999).
37. A. D. Watson, N. Leitinger, M. Navab, K. F. Faull, S. Horkko, J. L. Witztum, W. Palinski, D. Schwenke, R. G. Salomon, W. Sha, G. Subbanagounder, A. M. Fogelman and J. A. Berliner, *Journalof Bioogical Chemistry*, **272**, 13597 (1997).
38. F. Parhami, S. M. Jackson, Y. Tintut, V. Le, J. P. Balucan, M. C. Territo and L. L. Demer, *Journalof Bone Mineral Research*, **14**, 2067 (1999).
39. M. R. Brodeur, L. Brissette, L. Falstrault and R. Moreau, *Journal of Cellular Chemistry*, **105**, 1374 (2008).