Global Medical and Health Communication

Online submission: https://ejournal.unisba.ac.id/index.php/gmhc
DOI: https://doi.org/10.29313/gmhc.v12i2.13833

GMHC. 2024;12(2):126–130
pISSN 2301-9123 | eISSN 2460-5441

RESEARCH ARTICLE

Impact of Propolis Administration on Osteocrin Expression and Osteoblast-to-osteoclast Ratio in the Femurs of Rats Fed a High-fat Diet

Alvira Widiyanti,[1,2] Vita Murniati Tarawan,[3] Leonardo Lubis[4]

[1]Master of Biomedical Science Study Program, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia, [2]Faculty of Medicine, Universitas Islam Bandung, Bandung, Indonesia, [3]Department of Physiology, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia, [4]Department of Anatomy, Faculty of Medicine, Universitas Padjadjaran, Sumedang, Indonesia

Abstract

A high-fat diet (HFD) is associated with bone inflammatory processes that can affect bone remodeling balance. Osteocrin produced by periosteal osteoblasts correlates with osteoblast activity, is expressed on osteoblast-derived cells, and is localized in osteoblasts and young osteocytes. Propolis is an antioxidant and anti-inflammatory in bone remodeling by inhibiting proinflammatory factors NF-κB and COX-2, reducing inflammatory suppression of cytokines responsible for osteoclast differentiation and osteoblast apoptosis. The flavonoid content increases the production of nitric oxide and osteoprotegerin, which enhances osteoblastogenesis. This study determined the effect of propolis administration on bone formation and resorption in bone previously damaged by an HFD. This research was conducted in the Animal Laboratory of Postgraduate Building Dipati Ukur and Genetics and Molecular Laboratory Eycman Building, Faculty of Medicine, Universitas Padjadjaran. The research time was from January 2023–May 2024. Male Wistar rats were divided into four groups: normal chow diet (NCD), NCD with propolis administration, HFD, and HFD with propolis administration. The 12-week-old rats were given an HFD for 12 weeks and then treated with propolis at a 300 mg/kgBW dose for nine weeks. The administration of propolis increased the ratio of osteoblasts-osteoclast cells in the femur of the HFD rats but did not affect periosteal osteocrin expression.

Keywords: Bone, HFD, osteoblast, osteoclast, osteocrin

Introduction

The public's consumption of high-fat foods and beverages increases as they taste good.[1] A high-fat diet (HFD) is defined as consisting of at least 35% of the total calories consumed from unsaturated and saturated fat. High-fat diets influence the development of osteoporosis by affecting bone formation and resorption.[2] A high-fat environment can inhibit osteoblast proliferation and differentiation of other osteogenic cells, increase skeletal sclerostin expression, cause osteocyte lacunocanalicular damage, and increase local glucocorticoid signaling in bone, thereby affecting glucocorticoid signaling in osteoblasts and osteocytes. Bone marrow adipose tissue also showed decreased mRNA levels of inflammatory genes, such as TNF-α, IL-1β, and lipocalin 2.[2,3] Rats fed a high-fat diet had lower bone mineral content, and decreased structural parameters on histomorphometric examination by peripheral quantitative computed tomography analysis.[4]

Osteocrin is a humoral factor produced by periosteal osteoblasts that regulate growth plate growth by increasing the proliferation of C-type natriuretic peptide (CNP) to regulate bone formation and chondrocyte maturation.[5] In-vivo studies by Bord et al.[6] showed that osteocrin is a bone-active molecule expressed on osteoblast-derived cells, localized on osteoblasts and young osteocytes, and correlated with osteoblast activity.

Propolis is collected by honeybees and used for many purposes due to its antioxidant, anti-inflammatory, antimicrobial, anticancer, analgesic, antidepressant, and anxiolytic properties.[7] It contains more than 108 active compounds, including antioxidant flavonoids, which are recognized for their diverse health effects.[8,9] Flavonoids stimulate osteoblastogenesis by increasing nitric oxide and osteoprotegerin production and inhibiting

Received: 14 June 2024; Revised: 18 July 2024; Accepted: 22 July 2024; Published: 18 August 2024

Correspondence: Alvira Widiyanti. Master of Biomedical Science Study Program, Faculty of Medicine, Universitas Padjadjaran. Jln. Raya Bandung–Sumedang km 21, Sumedang 45363, West Java, Indonesia. E-mail: alvirawidiyanti@gmail.com

proinflammatory factors such as NF-κB and COX-2 that activate osteoclasts and thus stimulate osteoclastogenesis.[10,11] Propolis has been shown to enhance the bone remodeling process by reducing inflammatory suppression of cytokines responsible for osteoclast differentiation and osteoblast apoptosis.[12] A previous in-vivo study by Darmadi and Mustamsir[13] showed that propolis increased the number of osteoblasts and chondrocyte protein and decreased the number of osteoclasts in the femur of Wistar rats. Studies by Juwita et al.[14] showed an increase in the average ratio of osteoblasts to osteoclasts in the osteoporosis Wistar rats that were ovariectomized and given propolis orally. It indicates an increase in bone formation compared to the resorption due to a rise in osteoblasts and a decrease in osteoclasts. However, femoral metaphysis revealed that propolis does not affect cortical bone thickness. Therefore, this study was designed to determine the effect of propolis administration on bone formation and resorption in bone previously damaged by a high-fat diet.

Methods

The high-fat diet rat model derived from male 12-week-old Wistar rats was divided into four groups: normal chow diet (NCD), NCD with propolis administration, HFD, and HFD with propolis administration. The rats were fed an HFD (34.9% fat, 26.25% protein, and 26.3% carbohydrates) for 12 weeks, then treated with propolis at 300 mg/kgBW daily for nine weeks. Right and left femur bone samples were collected when the rats were 33–34 weeks old and kept in stored biological material.[15] This research was conducted in the Animal Laboratory of Postgraduate Building Dipati Ukur and Genetics and Molecular Laboratory Eycman Building, Faculty of Medicine, Universitas Padjadjaran. The research time was in January 2023–May 2024.

Osteocrin expression analysis using total RNA was extracted from stored left femur bone tissue using the Quick-RNA™ Miniprep-Kit and reverse transcribed using the SensiFAST cDNA synthesis kit according to manufacturer instructions. Osteocrin mRNA expression was quantified by real-time qPCR using the SensiFAST™ SYBR® No-ROX Kit.

Histological analysis of osteoblasts and osteoclast cells using right femur bone tissue that had previously been stored in 10% formalin was performed using right femur bone tissue that had previously been stored in 10% formalin. The tissue was decalcified in 8% HCl for ten weeks before being embedded in paraffin blocks and cut into four μm sections. The specimens were deparaffinized with xylene and rehydrated with graded alcohol before HE staining. The slides were examined using a Zeiss Image Z2 microscope at a magnification of 100× in three selected fields of view, and photomicrographs were taken of representative areas.

Data analysis is expressed as the mean±SD of triplicate measurements. The normality of the data was assessed using the Shapiro-Wilk test, with normally distributed data analyzed by one-way ANOVA and the Kruskall-Wallis test performed on data that is not normally distributed. The post hoc test was performed if the p-value was <0.05. Differences between groups were assessed using the independent t-test for normally distributed data and the Mann-Whitney test for data that were not normally distributed.

Ethical approval for this research was approved by the Research Ethics Committee of Universitas Padjadjaran (number 1456/UN6. KEP/EC/2023).

Results

There was no significant difference in osteocrin expression in the rat femur between the treatment groups (Table 1). Still, in general, HFD groups with or without propolis had lower median osteocrin expression than the NCD groups with or

Table 1 Osteocrin mRNA Expression in the Femur of Male Wistar Rats

Variables	NCD	NCD+propolis	HFD	HFD+propolis	p
Mean±SD	0.33±0.28	0.30±0.20	0.11±0.05	0.16±0.14	0.152[a*]
Median (min–max)	0.28 (0.06–0.74)	0.22 (0.14–0.73)	0.11 (0.04–0.19)	0.09 (0.04–0.41)	
Normality test[b]	0.263[+]	0.025[++]	0.243[+]	0.146[+]	

Note: n=3, [a]Kruskall-Wallis test, [b]Shapiro-Wilk test, [*]not significant, [+]normal data distribution, [++]abnormal data distribution

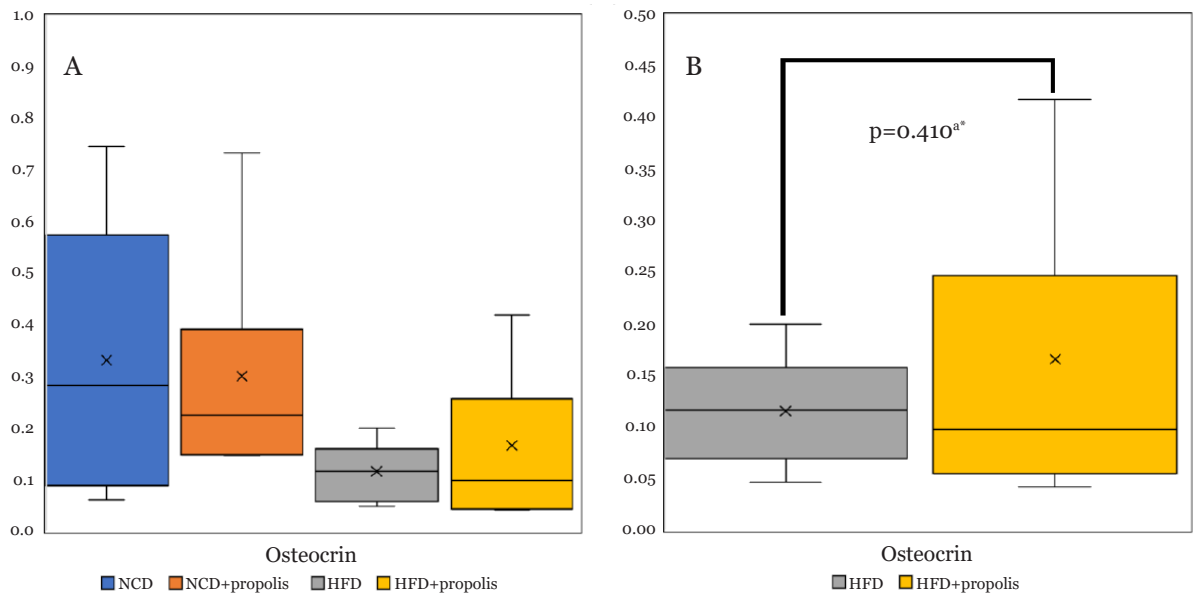

Figure 1 Comparison of Osteocrin mRNA Expression
Note: (A) four rat groups, (B) HFD groups, aindependent t-test, *not significant

without propolis (Figure 1A). Furthermore, there was a trend for increased osteocrin expression in the propolis-treated HFD rats compared to the HFD group, although this did not reach statistical significance (Figure 1B).

The osteoblast-osteoclast ratio in the femur of the four rat groups was significantly different with a p-value of 0.001 (Table 2), with the lowest ratio observed in the HFD groups (Figure 2A). An independent t-test of the HFD groups revealed that propolis significantly increased the test ratio (Figure 2B).

Discussion

A high-fat diet can lead to changes in bone structure due to a decreased number of osteoblasts and pre-osteoblasts, thus affecting bone remodeling. Progenitor cells undergoing adipogenesis can decrease progenitor cells, recruitment to osteoblastic cells, and bone formation. In addition, there is an increase in osteoporosis and increased osteoclast activity.[2,16]

Polyphenolic compounds in propolis act as antioxidants by inhibiting lipid peroxidation to protect bone and prevent excessive bone resorption, which can reduce bone density and strength through the clearance of reactive oxygen species. Flavonoids stimulate osteoblastogenesis by increasing nitric oxide and osteoprotegerin production and inhibiting proinflammatory factors such as NF-κB and COX-2 that activate osteoclasts and thus stimulate osteoclastogenesis.[10,11]

In this study, the lowest osteoblast-osteoclast ratios were observed in the femurs of the rats fed an HFD, with the highest ratio in the propolis-treated normal diet group. Although there was no significant difference in osteocrin expression between groups, the HFD groups tended to have the lowest osteocrin mRNA expression. It is hypothesized that the bone resorption caused by 12 weeks of HFD feeding and bone remodeling caused by four weeks of propolis treatment had no

Table 2 Osteoblast-osteoclast Ratio in the Femur of the Four Rat Groups

Variables	NCD	NCD+propolis	HFD	HFD+propolis	p
Mean±SD	7.96±2.33	12.37±7.24	3.11±0.98	4.84±1.67	0.001[a*]
Median (min–max)	6.92 (6.02–11.67)	11.30 (0–23.24)	2.83 (2.01–4.76)	4.51 (2.50–8.00)	
Normality test[b]	0.12[+]	0.46[+]	0.35[+]	0.49[+]	

Note: [a]One-way ANOVA test, [b]Shapiro-Wilk test, [*]significant, [+]normal data distribution

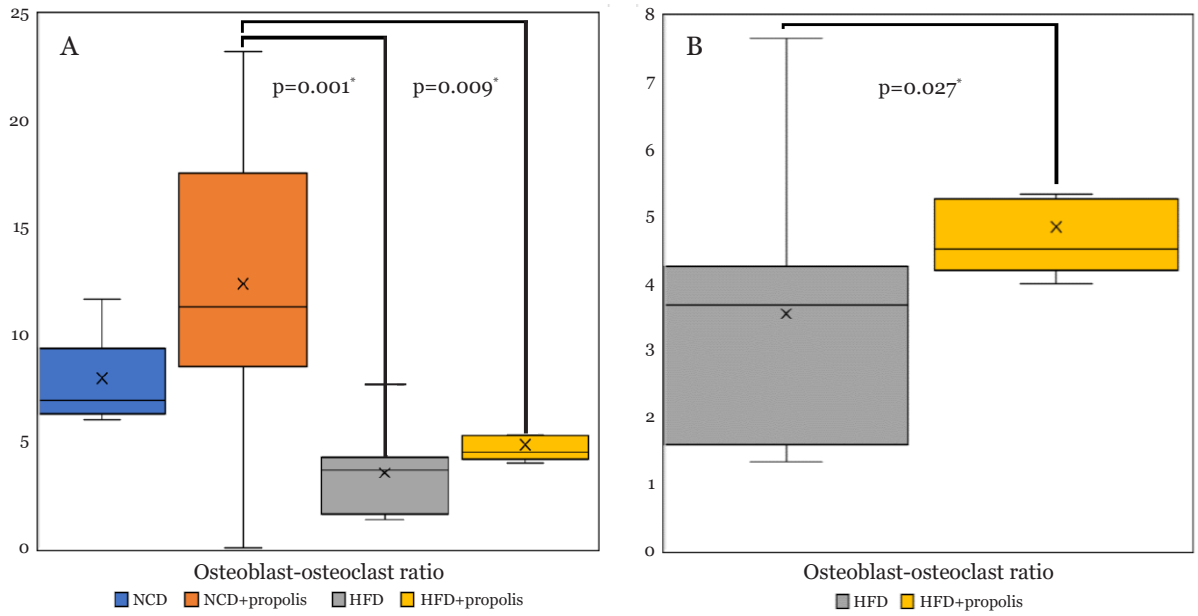

Figure 2 Comparison of the Osteoblast-osteoclast Ratio
Note: (A) four rat groups, (B) HFD groups, independent t-test, Mann-Whitney test, *significant p<0.05

observable effect on the periosteum because these processes begin in the bone endosteum, whereas osteocrin is in the periosteum. The periosteum is the outermost fibrous layer that maintains the bone structure, and the cambium layer contains osteoprogenitor cells. The endosteum layer consists of connective tissue, osteoblasts, pre-osteoblasts, and osteoclasts and is involved in bone repair and remodeling.[17] Osteocrin produced by periosteal osteoblasts is a mechanotransducer that regulates growth plate growth by increasing chondrocyte proliferation and maturation for bone elongation. CNP stimulates the osteogenic differentiation of periosteal osteoprogenitors to induce bone formation.[18]

In this study, the rats were terminated at 31–33 weeks of age, which reflects 24–25 years in humans. It is suspected that at this age, the process of bone elongation has decreased along with the closure of the epiphyseal plate. Rat regrowth peaks at 4–6 months of age.[19,20] Osteocrin for bone lengthening correlates with increasing CNP. Osteocrin expression is localized in osteoblasts and young osteocytes during bone formation and development and will decrease with age.[18]

Conclusion

The administration of propolis increases the ratio of osteoblasts to osteoclasts in the femur of rats fed a high-fat diet.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

The authors thank the supervisors for their contributions to this study.

References

1. Wang T, Zhu X, Dai F, Li C, Huang D, Fang Z, et al. Effects of a standard high-fat diet with or without multiple deficiencies on bone parameters in ovariectomized mature rat. PLoS One. 2017;12(9):e0184983.
2. Zhu R, Wang Z, Xu Y, Wan H, Zhang X, Song M, et al. High-fat diet increases bone loss by inducing ferroptosis in osteoblasts. Stem Cells Int. 2022;2022:9359429.
3. Duan W, Wang Q, Li F, Xiang C, Zhou L, Xu J, et al. Anti-catabolic effect of caffeic acid phenethyl ester, an active component of honeybee propolis on bone loss in ovariectomized mice: a micro-computed tomography study and histological analysis. Chin Med J (Engl). 2014;127(22):3932–6.

4. Graham LS, Tintut Y, Parhami F, Kitchen CM, Ivanov Y, Tetradis S, et al. Bone density and hyperlipidemia: the T-lymphocyte connection. J Bone Miner Res. 2010;25(11):2460–9.
5. Kanai Y, Yasoda A, Mori KP, Watanabe-Takano H, Nagai-Okatani C, Yamashita Y, et al. Circulating osteocrin stimulates bone growth by limiting C-type natriuretic peptide clearance. J Clin Invest. 2017;127(11):4136–47.
6. Bord S, Ireland DC, Moffatt P, Thomas GP, Compston JE. Characterization of osteocrin expression in human bone. J Histochem Cytochem. 2005;53(10):1181–7.
7. Hossain R, Quispe C, Khan RA, Saikat ASM, Ray P, Ongalbek D, et al. Propolis: an update on its chemistry and pharmacological applications. Chin Med. 2022;17(1):100.
8. Farida S, Pratami DK, Sahlan M, Laksmitawati DR, Rohmatin E, Situmorang H. In-vitro antioxidant, in-vivo anti-inflammatory, and acute toxicity study of Indonesian propolis capsule from *Tetragonula sapiens*. Saudi J Biol Sci. 2022;29(4):2489–500.
9. Segueni N, Zellagui A, Moussaoui F, Lahouel M, Rhouati S. Flavonoids from Algerian propolis. Arab J Chem. 2016;9(Suppl 1): S425–8.
10. Minematsu A, Nishii Y, Sakata S. High-fat/high-sucrose diet results in higher bone mass in aged rats. Bone Rep. 2018;8:18–24.
11. Mazur CM, Castro Andrade CD, Tokavanich N, Sato T, Bruce M, Brooks DJ, et al. Partial prevention of glucocorticoid-induced osteocyte deterioration in young male mice with osteocrin gene therapy. iScience. 2022;25(9):105019.
12. Ekeuku SO, Chin K. Application of propolis in protecting skeletal and periodontal health—a systematic review. Molecules. 2021;26(11):3156.
13. Darmadi D, Mustamsir E. The effect of propolis on increasing the number of osteoblasts and chondrocytes, and decreasing the number of osteoclasts in Wistar rats (*Rattus novergicus*) with femoral bone fracture. IOSR J Dent Med Sci. 2016;15(12):90–5.
14. Juwita DA, Ahmadi A, Rachmaini F, Abdillah R, Fatma RM. Effect of propolis on bone quality and cortical bone thickness of ovariectomized female Wistar white rats as a model for osteoporosis. Pharm Sci Res. 2021;8(3):121–7.
15. Zheng Y, Wu Y, Tao L, Chen X, Jones TJ, Wang K, et al. Chinese propolis prevents obesity and metabolism syndromes induced by a high fat diet and accompanied by an altered gut microbiota structure in mice. Nutrients. 2020;12(4):959.
16. Duan Y, Zeng L, Zheng C, Song B, Li F, Kong X, et al. Inflammatory links between high fat diets and diseases. Front Immunol. 2018;9:2649.
17. Moore KL, Dalley AF, Agur AMR. Clinically oriented anatomy. 7th edition. Baltimore: Lippincott Williams & Wilkins; 2014.
18. Watanabe-Takano H, Ochi H, Chiba A, Matsuo A, Kanai Y, Fukuhara S, et al. Mechanical load regulates bone growth via periosteal osteocrin. Cell Rep. 2021;36(2):109380.
19. McWain MA, Pace RL, Nalan PA, Lester DB. Age-dependent effects of social isolation on mesolimbic dopamine release. Exp Brain Res. 2022;240(10):2803–15.
20. Nguyen-Yamamoto L, Tanaka KI, St-Arnaud R, Goltzman D. Vitamin D-regulated osteocytic sclerostin and BMP2 modulate uremic extraskeletal calcification. JCI Insight. 2019;4(13):e126467.