THE BONES STRENGTH EFFECT OF PROPOLIS IN OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

Dian Ayu Juwita[*], Almahdy A[*], Rahmad Abdillah, [*] Fiony Syahputri [*]

*Departement Pharmacology, Faculty of Pharmacy, Andalas University,

Padang, West Sumatera, Indonesia

Corresponding author	:	Dian Ayu Juwita
Email	:	dianayujuwita@phar.unand.ac.id
Phone number	:	+62 813 6300 1618
Affiliation	:	Departement Pharmacology, Faculty of Pharmacy, Andalas University, Padang, West Sumatera, Indonesia
Address	:	Departement Pharmacology Faculty of Pharmacy building, Andalas University, Limau Manis , Padang, West Sumatera, Indonesia, Postcode 25163

Conflicts of Interest Statement

Manuscript title: THE BONES STRENGTH EFFECT OF PROPOLIS IN OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to affect the objectivity and credibility of the work reported in this paper.

- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This statement is signed by all the authors to indicate agreement that the above information is true and correct

Padang, September 16, 2020

Dian Ayu Juwita | Almahdy. A | Rahmad Abdillah | Fiony Syahputri

Authorship Conformation

Manuscript title: THE BONES STRENGTH EFFECT OF PROPOLIS IN OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

All authors have participated in
(a) conception and design, or analysis and interpretation of the data;
(b) drafting the article or revising it critically for important intellectual content; and
(c) approval of the final version.

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

This statement is signed by all the authors to indicate agreement that the above information is true and correct

Padang, September 16, 2020

Dian Ayu Juwita	Almahdy. A	Rahmad Abdillah	Fiony Syahputri

Statement of the sources of funding

Manuscript title: THE BONES STRENGTH EFFECT OF PROPOLIS IN OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

The authors are thankful to Faculty of Pharmacy, Andalas University, which has provided financial support and facilities for conducting this research

INTRODUCTION

Osteoporosis is a disease characterized by abnormalities of bone tissue and changes in bone microarchitecture that can affect the strength of bone impact and cause fractures (1). This disease often occurs especially in women who are menopausal and elderly people. Two out of five women in Indonesia have a greater risk of osteoporosis (2,3).

Women have a risk of decreasing bone mass faster than men (4,5). This is due to the reduced production of the hormone estrogen especially in women who have experienced menopause (5,6). This estrogen hormone is very necessary in the formation of osteoblasts and prevention of the activity and division of osteoclast cells with its activity against osteoclast receptors(7,8).

One of the commonly used herbal medicines is propolis. Propolis is a natural resin or gum substances collected by bees from exudates of certain tree species that can determine the chemical composition of propolis. One of its pharmacological effects can prevent osteoporosis (9). The main components of flavonoids and phenolic acids in propolis are known can protect bone health (10).

Flavonoid compounds contained in propolis include pinocembrin, quercetin, naringin, galanin, and chrysin. Whereas phenolic acid, namely Caffeic Acid Phenethyl Ester (CAPE) (11). Flavonoids can protect bone health by five reaction mechanisms, namely reducing bone resorption through antioxidant activity, anti-inflammatory activity, increased osteoblastogenesis activity, suppress osteoclastogenesis activity, and increase its activity in osteo immunologicals (12). Flavonoid activity of antioxidants, namely flavonoids, will increase the differentiation of osteoblasts and decrease apoptosis from these cells by increasing bone-forming enzymes, namely Alkaline Phosphatase, collagen I, and other bone matrix proteins. In osteoclasts, flavonoids will reduce its differentiation and increase its apoptosis by reducing the amount of RANKL, reducing the amount of Acid Phosphatase and

Cathepsin K protease. In osteocyte cells, flavonoid compounds will decrease RANKL, increase the production of OPG compounds, decrease Sclerostin and Dicckoph (13). High number of osteoclasts results in the risk of osteoporosis (14)

Propolis is easily found in Indonesian nature. The use of propolis as herbal medicines is very effective and widely used. However, for osteoporosis effect, the research is still limited. So it is necessary to do anti-osteoporosis research from propolis with various doses of female white rats that are ovariectomized by measuring the strength of bone impact using the Impact Testing Machine.

MATERIALS AND METHODS

This study was conducted at the Pharmacology Laboratory of the Faculty of Pharmacy and the Mechanical Metallurgy Laboratory of the Department of Mechanical Engineering, Faculty of Engineering, Andalas University, Padang, West Sumatra.

The experimental animals used were 25 female white Wistar rats with 150-200 gram weight and 3 months old. Ethical clearance of this research has been approved by The Committee of the Research and Ethic in Faculty of Medicine, Andalas University. The sample used is the brand "X" propolis concentration of 150 mg / mL with different doses.

Animals were acclimatized for 7 days, then divided into five treatment groups. Group I were ovariectomy rats and administered propolis at a dose of 180 mg/kg BW. Group II were ovariectomy rats and administered propolis at a dose of 360 mg/kg BW, Group III were ovariectomy rats and administered propolis at a dose of 720 mg/kg BW. Group IV were the negative control, only given food and drink. Group V were the positive control, ovariectomy is performed, without administrated of propolis. All group were treated for 30 days.

The impact strength of the bone were measured with the Impact Testing Machine Setra BL-4100L. The impact strength is calculated by comparing the impact energy obtained

with the cross-sectional area of the sample that was previously measured. All data will be analysis with one-way ANOVA.

RESULTS AND DISCUSSION

The results obtained from the rat bone impact strength as shown in table 1. The analysis obtained a significance value of P <0.05 meaning that there are significant result in treatment groups.

Based on the results, we found that the average impact strength is quite diverse. In the negative control group, the mean value of bone impact strength showed the highest number which was 3.9655 J/mm^2. Whereas in the positive control group, the value of bone impact strength, in general had the lowest value of 3.2231 J/mm^2.

In the treatment group with a dose of 180 mg/kg body weight, 360 mg/kg body weight, and 720 mg/kg body weight the average strength of bone impact values was 3,2901 J/mm^2, 3,4969 J/mm^2, and 3, 8105 J/ mm^2 (Table 2 and figure 1).

To see the difference in each treatment group, then followed by Duncan's test. In Duncan's further tests as seen in table 3, three different subsets were obtained. In the first subset there was a positive control group, a dose group of 180 mg/kg body weight and a dose group of 360 mg/kg body weight with a significant value of 0.215 (P> 0.05). In subset two there was a dose group of 360 mg/kg body weight and a dose group of 720 mg/kg body weight with a significant value of 0.135 (P> 0.05). In subset three there was a dose of 720 mg/kg BW and negative control, meaning that the two treatment groups were not significantly different with a significant value of 0.450 (P> 0.05).

Based on the results of observations from the average impact strength values we obtained quite a diverse data between the types of treatment given. From these values, it can be concluded that there are differences in the average value of bone impact strength from negative controls, a dose of 180 mg/kg BW, a dose of 360 mg/kg BW, a dose of 720 mg/kg

BW and positive control. The value of bone impact strength is influenced by the physiology of the body, bone size, number of bone-forming cells, and the number of minerals in bone from experimental animals.

In bone impact strength testing using an impact testing machine, there were differences in the average impact strength values of various test groups with the control group. In the negative control group (not on ovariectomy and not given propolis) the average value of bone impact strength showed the highest rate of 3.9655 J/ mm^2. This is because the experimental animals used were not in menopause condition, where the hormone estrogen is still produced by the ovaries, so the highest bone impact strength values are generally found in this group. While in the positive control group (in ovariectomy but not given propolis) the value of bone impact strength, in general, has the lowest value of 3.2231 J/mm^2. According to Mustafa S (2011), this is caused by a decrease in estrogen hormone due to menopause which is one of the factors that trigger osteoblast cell formation. In postmenopausal women, estrogen hormone deficiency occurs, where this hormone serves as a trigger for the formation of osteoblasts in the myeloid tissue of red marrow in adult individuals(15). Bone strength is influenced by the bone quality, bone quantity and bone remodelling process played by osteoclasts and osteoblasts(16).

In the treatment group with a dose of 180 mg/kg BW, an average bone impact strength of 3.2901 J/mm^2 was not significantly different from positive control, where there was only a slight increase in bone strength when propolis was administered. According to Domazetovic (2017) propolis works as an antioxidant which has the opposite effect of Reactive Oxidative Stress (ROS), where antioxidants increase the amount of osteoblast formation (13).

At a dose of 360 mg/kg BW group, the average value of bone impact strength is 3.4969 J/mm^2. This dose is a general dose used to treat osteoporosis, where the value of bone impact strength is quite different compared to positive control and the dose is 180 mg/kg body

weight so that it has a fairly maximal effect. In postmenopausal women, the amount of estrogen will decrease because the ovaries which are one of the producers of this hormone are no longer functioning. The hormone estrogen plays a role in maintaining the balance of the number of osteoblasts and osteoclasts in the bone (17).

At a dose of 720 mg/kg BW which is the highest dose given shows a significantly higher impact strength value of 3.8105 J/mm^2 than the positive control and also higher than a dose of 180 mg/kg BW and 360 mg/kg BW but lower than negative controls. At a dose of 720 mg/kg BW this protective effect is seen in maintaining the balance of bone remodelling so that the process of the bone formation increases. This can be seen from its value which is almost close to the negative control. Estrogen plays a very important role in bone metabolism by maintaining the balance of bone-forming cells (osteoblasts) and bone-destroying cells (osteoclasts) (18)

In research conducted by Al-Qtaitat (2014), the administration of propolis can maintain the stability of the skeletal bones of experimental animals that are ovariectomized. This is because the flavonoid compounds in propolis have characteristics as selective estrogen receptor modulators (SERMs) that can prevent bone resorption by osteoclasts and increase the formation of osteoblasts so that they can prevent osteoporosis (9). The bone impact strength is influenced by mineral content in bone, bone size, bone structure, and balance of bone remodelling by osteoblasts and osteoclasts (19).

In Duncan's further tests, three different subsets were obtained. Where, if the treatment group is in the same subset it means that the group is not significantly different from the P value> 0.05. Whereas in the subset it was seen that a significant difference was found in doses of 180 mg/kg body weight and doses of 720 mg/kg body weight, meaning that the effect of these two treatment groups was significantly different on the strength of the rat bone impact.

Bone strength and hardness are also determined by bone inorganic components such as phosphorus, carbonic acid, etc. An increase in the impact strength of femur bones in experimental animals given propolis at different doses is caused by the content of flavonoids and polyphenols which have antioxidant activity (20). Flavonoids in propolis like pinocembrin, naringenin, CAPE, myricetin, etc. These flavonoid compounds have antioxidant activity as opposed to ROS, where antioxidants will increase osteoclastogenesis (21).

CONCLUSION

Administration of propolis at a dose of 180 mg/kg BW, a dose of 360 mg/kg BW, and a dose of 720 mg/kg BW have the bones strength effect on femur bones in female white rats as a postmenopausal ovariectomized model.

REFERENCES

1. Ohta H, Solanki J. Incorporating bazedoxifene into the treatment paradigm for postmenopausal osteoporosis in Japan. Osteoporos Int. 2015;26(3):849–63.
2. Sozen T, Ozisik L, Calik Basaran N. An overview and management of osteoporosis. Eur J Rheumatol. 2017;4(1):46–56.
3. Mohd Effendy N, Mohamed N, Muhammad N, Mohamad IN, Shuid AN. The effects of Tualang honey on bone metabolism of postmenopausal women. Evidence-based Complement Altern Med. 2012;2012(June 2014).
4. Ermawati DH. Menopause and Biopsychosocial Factors Associated with Quality of Life in Women in Surakarta, Central Java. J Matern Child Heal. 2018;03(02):119–27.
5. Gambacciani M, Biglia N, Cagnacci A, Caruso S, Cicinelli E, De Leo V, et al. Menopause and hormone replacement therapy: The 2017 recommendations of the Italian menopause society. Minerva Ginecol. 2018;70(1):27–34.
6. Thulkar J, Singh S. Overview of research studies on osteoporosis in menopausal women since the last decade. J Midlife Health. 2015;6(3):104.
7. Khosla S, Oursler MJ, Monroe DG. Estrogen and the skeleton. Trends in Endocrinology and Metabolism. 2012;23(11):576–81.
8. Stavros C M, O'Brien CA, Almeida M. The role of estrogen and androgen receptors in bone health and disease. Nat Rev Endocrinol [Internet]. 2013;9(12):699–712. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf
9. Al-Qtaitat A. Bioactive Propolis and Bone Loss Reduction in an Ovariectomized Rat Model of Hypogonadal Osteoporosis. Am J Biosci. 2014;2(6):217.
10. Wagh VD. Propolis: A wonder bees product and its pharmacological potentials. Adv Pharmacol Sci. 2013;2013.
11. Kuropatnicki AK, Szliszka E, Krol W. Historical aspects of propolis research in

modern times. Evidence-based Complement Altern Med. 2013;2013(November 2015).

12. Dudarić L, Fužinac-Smojver A, Muhvić D, Giacometti J. The role of polyphenols on bone metabolism in osteoporosis. Food Res Int. 2015;77:290–8.

13. Domazetovic V, Marcucci G, Iantomasi T, Brandi ML, Vincenzini MT. Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab. 2017;14(2):209.

14. Florencio-Silva R, Rodrigues da Silva Sasso G, Sasso-Cerri E, Jesus Simões M, Sérgio Cerri P. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Hindawi Publ Corp BioMed Res Int. 2015;7(1):1–17.

15. Singh D, Sanyal S, Chattopadhyay N. The role of estrogen in bone growth and formation: Changes at puberty. Cell Health Cytoskelet. 2011;3(1):2–12.

16. Tavera Ruiz CG, De La Torre-Ibarra MH, Flores-Moreno JM, Frausto-Reyes C, Mendoza Santoyo F. Cortical bone quality affectations and their strength impact analysis using holographic interferometry. Biomed Opt Express. 2018;9(10):4818.

17. Puspita EM, Siregar MFG, Adenin I. Correlation of Estradiol Serum Levels with Classification of Osteoporosis Risk OSTA (Osteoporosis Self-Assessment Tools for Asian) in Menopause Women. Bali Med J. 2017;6(1):52.

18. Nur H, Toraman NF, Arica Z, Sarier N, Samur A. The relationship between body composition and bone mineral density in postmenopausal Turkish women. Rheumatology International. 2013; 33: 607–612.

19. Yudaniayanti IS, Primarizky H, Nangoi L, Yuliani GA. Protective effects of honey by bees (Apis dorsata) on decreased cortical thickness and bone impact strength of ovariohysterectomized rats as models for menopause. Vet World. 2019;12(6):868–76.

20. An J, Hao D, Zhang Q, Chen B, Zhang R, Wang Y, et al. Natural products for treatment of bone erosive diseases: The effects and mechanisms on inhibiting

osteoclastogenesis and bone resorption. Int Immunopharmacol. 2016;36(July):118–31.

21. Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. Propolis: A Complex Natural Product with a Plethora of Biological Activities That Can Be Explored for Drug Development. Evidence-based Complement Altern Med. 2015: 1-29.

THE BONES STRENGTH EFFECT OF PROPOLIS IN OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

Dian Ayu Juwita[*], Almahdy A[*], Rahmad Abdillah, [*] Fiony Syahputri [*]

Departement Pharmacology Faculty of Pharmacy, University of Andalas,

Padang, West Sumatera, Indonesia

ABSTRACT

Osteoporosis is a bone disease characterized by decreased quality and strength of bones so that it becomes porous and fracture. Propolis is known to have many pharmacological activity including anti-osteoporosis effect. This study aims to determine the effect of propolis administration and the effects of propolis dosage variation in preventing osteoporosis based on the strength value of femur bone impact in female white rats as an ovariectomy postmenopausal modelling. The rats were divided into 5 groups: positive control group, negative control group, and treatment group that were ovariectomy and given propolis at a dose of 180 mg/kg BW, dose 360 mg/kg BW and dose 720 mg/kg BW. Propolis was given orally for 30 days. Bone impact strength testing is done after 30 days using an impact testing machine. Research data were analyzed with one-way ANOVA and continued with the Duncan's Multiple Range Test. From the test results, it was found that propolis had a significant effect on the value of bone strength, with the dose of 720 mg/kg BW and 360 mg/kg BW had a significant effect, compared with others. With an increase in dose, propolis can provide an increase in the value of bone strength in rat bones compared with the positive control group.

Keywords: Osteoporosis, propolis, impact strength, menopause, ovariectomy