EFFECT OF PROPOLIS ON BONE IMPACT STRENGTH OF OVARIECTOMIZED FEMALE WHITE RATS AS MODELS FOR POSTMENOPAUSE

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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, white rats, ovariectomy

1. 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] Osteoporosis is also known as "silent disease " which can increase in certain age groups which can lead to death.[2]

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 substance collected by bees from exudates of certain tree species that can determine the chemical composition of propolis.[9] 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 1.[13] The 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.

Methodology

The 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.

Experimental animals and sample

The 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 Ethics of the Faculty of Medicine, Andalas University, Padang which number 146/KEP/FK/2020

The sample used is the brand "X" propolis concentration of 150 mg / mL with different doses.

Preparation of Animals

Animals were acclimatized for 7 days, then divided into five treatment groups.

1. Group I: ovariectomy and was given induction of propolis at a dose of 180 mg/kg BW for 30 days.
2. Group II: ovariectomy and was given induction of propolis at a dose of 360 mg/kg BW for 30 days.
3. Group III: ovariectomy and given induction of propolis at a dose of 720 mg/kg BW for 30 days.
4. Group IV (negative control): only given food and drink.
5. Group V (positive control): ovariectomy is performed, propolis induction is not given.

Ovariectomy

1. Mice fasted for 6 hours.
2. Give ketamine anesthesia 50 mg / kg BW and xylazine 10 mg / kg BW intraperitoneally (IP).
3. Place the mouse in a supine position.
4. Shave the mouse hair around the area to be operated on, namely the left flank area with a length of ± 15 cm, after that wet the cotton with soap and wash off the shaved area.
5. Clean the area that has been shaved with clean cotton, then spray 70% alcohol.
6. Give sterile duk in the area that has been sprayed with 70% alcohol and with sterile gauze rub povidone-iodine to the surgical area counterclockwise from the inside out.
7. Make an incision in the skin, subcutaneous section and in the alba line from cranial to caudal direction with a length of ± 4 cm.
8. Leave the incision open, remove the left and right ovaries.
9. The cut cervical tip is inserted back into the abdomen.
10. Sew the alba line back, then finish with subcutan sewing and do the outer stitch.
11. Give gentamicin antibiotics to avoid infection.
12. Observe animals for 1 day to heal cuts.

Impact strength testing

After being given propolis induction for 30 days, the animals were euthanasia and then necropted, namely the removal of the right femur bone to be analyzed for bone strength with the Impact Testing Machine brand Setra BL-4100L. First, the femur bone that has been necropted is preserved in formalin 10%. Sample preparation begins by measuring the diameter of the bone to find the cross-sectional area of the sample. The bone sample is then inserted into the desiccator with 50% ± 5 RH for 40 hours. Bone samples are placed in the workplace so that the sample can stand upright, then the pendulum or the load to be applied is suddenly raised to an angle of α (160 °) as the initial height. Press the pendulum retraction lever so that the pendulum will swing about the sample until it breaks, then the value of the impact energy can be seen on the monitor. The impact strength is calculated by comparing the impact energy obtained with the cross-sectional area of the sample that was previously measured.

Impact Strength=

Data analysis

Data normality test with Kolmogorov-Smirnov test, homogeneity test data with Levene test then bone strength data from impact strength analysis in each treatment group will be seen the difference with one-way ANOVA analysis.

Result

From the femur bone impact strength testing conducted on 25 female white rats after being given propolis for 30 days, the results obtained from the rat bone impact strength as shown in table 1. The data obtained were then analyzed with SPSS version 25 which was started with a data normality test using the Kolmogorov-Smirnov and Shapiro-Wilk test. The results of normality test data on the impact strength parameter values for each type of treatment obtained normally distributed data (there is no real difference between variants) because in all parameters obtained values of P> 0.05. Then the data homogeneity test is performed using the Levene test. Homogeneity test results showed a value of P> 0.05, where Ho is accepted, which means that the variants of the five groups are the same or homogeneous, so it can be continued

with the ANOVA test. Then proceed with the one-way ANOVA test to see differences between variants. The analysis obtained a significance value of P <0.05 meaning that there are significant differences between treatment groups.

Table 1. Results of impact strength femur bone impact strength of female white rats after being given propolis for 30 days

Treatment Group	Impact Strength of Bone (J/mm^2)				
	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5
Dose 180 mg/kg BB	3,2513	3,3201	2,9586	3,5735	3,3470
Dose 360 mg/kg BB	3,2749	3,7658	3,4568	3,4827	3,5044
Dose 720 mg/kg BB	3,6364	3,9966	3,6316	3,7607	4,0270
Negative control	4,3808	4,2454	4,0286	3,4327	3,7399
Positive control	3,4568	3,3867	3,7384	2,4422	3,0982

Based on the results of the study, 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 as shown in table 2 and figure 1.

Table 2. Mean value of femoral bone impact strength of female white rats from the five treatment groups

Treatment Group	Mean value of Impact Strength ± SE
Dose 180 mg/kg BB	3,2901 ± 0,2213^{a}
Dose 360 mg/kg BB	3,4969 ± 0,1757^{ab}
Dose 720 mg/kg BB	3,8105 ± 0,1912^{bc}
Negative control	3,9655 ± 0,3839^{c}
Positive control	3,2231 ± 0,4925^{a}

Note: [abc] is a different superscript in the same column

Figure 1. A bar chart of the average strength value of femur females rat bone impact strength from the five treatment groups

To see the difference in each treatment group, then followed by Duncan's test. In Duncan's further tests, three different subsets were obtained. In subset one there is 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 is 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).

Table 3. Duncan's further test results on the effect of propolis dose given with femoral bone impact strength in female white rats

Treatment Groups	N	Subset for alpha = 0.05		
		1	2	3

Positive control	5	3.2245		
Dose 180 mg/kg BB	5	3.2901		
Dose 360 mg/kg BB	5	3.4969	3.4969	
Dose 720 mg/kg BB	5		3.8105	3.8105
Negative control	5			3.9655
Sig.		.215	.135	.450

Discussion

Based on the results of observations of the average impact strength values in test animals obtained quite 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 did not experience menopause, where the hormone estrogen is still produced by

the ovaries, so the highest bone impact strength values are generally found in female white rats used in this study. 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 the hormone estrogen 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.[16] Bone strength is influenced by the bone quality, bone quantity and bone remodelling process played by osteoclasts and osteoblasts.[17]

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 given propolis and was the lowest dose given. 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 body weight, 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. According to Puspita EM (2017) 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.[18]

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. According to Humaryanto (2017), estrogen plays a very important role in bone metabolism by maintaining the balance of bone-forming cells (osteoblasts) and bone-destroying cells (osteoclasts) .[19]

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] According to Yudaniayanti (2019) states that bone impact strength is influenced by mineral content in bone, bone size, bone structure, and balance of bone remodelling by osteoblasts and osteoclasts.[20]

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.[21] 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.[22] Flavonoids in propolis like pinocembrin, naringenin, CAPE, myricetin, etc. These flavonoid compounds have antioxidant activity as opposed to ROS, where antioxidants will increase osteoclastogenesis.[23]

Conclusion

Provision of propolis at a dose of 180 mg/kg body weight, a dose of 360 mg/kg body weight, and a dose of 720 mg/kg body weight affected in preventing a decrease in the impact strength of femur bones of female white rats as a postmenopausal model that was ovariectomized.

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Conflict of interest

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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, Basaran NC. An overview and Management of Osteoporosis. European Journal of Hematology. 2017;4:46-56
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;2012:7.
4. Ermawati DH, Budihastuti UR, Murti B. Menopause and Biopsychosocial Factors Associated with Quality of Life in Women in Surakarta, Central Java. J Maternal and Child Health. 2018;3(2):119-127
5. Hans S, Hans S. Menopause And Hrt. Self Assess Rev Gynecol. 2017;(April):44–44
6. Thulkar J and Singh S. Overview of Research Studies on Osteoporosis in Menopausal since the Last Decade. J of Mid Life Health. 2015;6(3):104-107.
7. Khosla S, Oursler MJ, Monroe DG. Estrogen and the Skeleton. Trends Endocrinol Metab. 2012;23(11):576-581
8. Sihombing I, Wangko S, Kalangi Sjr. The role of Estrogen in Bone Remodelling. J Biomedik. 2013;4(3)
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;12
11. Kuropatnicki Ak, Szliszka E, Krol W. Historical Aspects Of Propolis Research In Modern Times. Evidence-Based Complement Altern Med. 2013;2013:12
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):209–16

14. Silva RF, Rodrigues G, Cerri ES, Simoes MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors that Influence Bone Cell. 2015;1-17
15. Laksmi. The Relationship of Body Composition with Bone Density of Fertile Age Women in Bandung City. Indones J Hum Nutr [Internet]. 2018;1(1):23–34
16. Mustafa S, Sigit K, Priosoeryanto Bp, Manalu W, Anatomi L, Hewan Fk, Et Al. Quality of Normal Female Mouse Bones that were Extracted by Wetah Extract During Growth. J Vet. 2012;12(2):113–9
17. 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
18. 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
19. Humaryanto H. Early Detection of Postmenopausal Osteoporosis. Jambi Med J. 2017;5(2):164–77
20. 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
21. Adaptation B, Impact To, Of S, Intensity L. Bone Adaptation To Impact Loading—Significance Of Loading Intensity. Medicine. 2007
22. 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 [Internet]. 2016;36:118–31
23. 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;2015:29