Nat. Volatiles & Essent. Oils, 2021; 8(4): 10489-10503

NVEO
Natural Volatiles & Essential Oils

The Effects of Green Bean Extract on Serum Calcium and Mandibular Bone Calcium Levels in Menopausal Rat Model

Endah Aryati Eko Ningtyas[1,2] , Oedijani-Santoso[3] , Udadi Sadhana[4] , Siti Sunarintyas[5]

[1]Doctoral Program of Medical and Health Science, Faculty of Medicine Diponegoro University Semarang, Jln. Prof. Soedarto, SH,Tembalang, Semarang, Indonesia, Postcode 1269

[2]Poltekkes Kemenkes of Semarang, Jl Tirto Agung, Pedalangan, Banyumanik, Semarang, Central Java Postcode 50268

[3]Dentistry Study Program, Medical Faculty, Diponegoro University, Jalan Prof.Soedarto, SH Tembalang, Semarang,Indonesia, Postcode1269

[4]Anatomy Pathology Department, Medical Faculty, Diponegoro University, Jalan Prof. Soedarto, SH Tembalang, Semarang,Indonesia, Postcode1269

[5]Dental Biomaterial Department, Dentistry Faculty, Gadjah Mada University, Jalan Denta Sekip Utara, Yogyakarta, Indonesia,Postcode55581

.Email:endahaeyatu@gmail.com

Abstract

Osteoporosis is a decrease in bone mass experienced by many menopausal women and becomes a "silent killer" or a silent disease for them. The green bean extract may be able to act as a phytoestrogen and a source of calcium to prevent osteoporosis caused by estrogen deficiency and a lack of calcium intake. However, it has been explored deeply. This study is aimed to know the effect of green bean extract on serum and mandibular bone calcium levels of the menopausal model. This study is an experimental laboratory study with Post Test Only Control Group Design, using female Wistar rats and divided into 5 (five) groups: baseline (C1); without ovariectomy and green bean extract (C2); ovariectomy without green bean extract (T1); ovariectomy and green bean extract (T2); ovariectomy and treated with bisphosphonates (T3). Serum and bone calcium levels were analyzed using atomic absorbance spectrophotometric (AAS). The results show that the control (C) and treatment (T3) groups had significant differences in bone calcium levels in the treatment group (T1 and T2) ($p<0.05$). The T1 group had a significant difference from T2. The results also show that the treatment had a weak relationship with serum calcium levels ($r=0.376$; $p=0.064$) but strongly correlated with bone calcium ($r=0.438$; $p=0.029$). Meanwhile, bone calcium had a negative correlation with serum calcium levels; the higher the serum calcium level, the lower the bone calcium ($r=-0.437$; $p=0.029$).

Green bean extract reduces serum calcium levels and increased mandibular bone calcium levels in experimental menopausal models.

Keywords: green bean, serum calcium level, mandibular bone calcium

Introduction

Osteoporosis is a decrease in bone mass due to an imbalance in the bone remodeling process when bone resorption activity is higher than bone apposition. Osteoporosis is experienced by many menopausal women and is a "silent killer" or silent disease for them. This is because most women are not aware of suffering from this disease untilserious bone pain and fractures occur (Jahan,2015; Szamatowicz, 2016). 1 in 3 women in the world over 50 years of age will experience a fracture due to osteoporosis where this fracture incidence occurs 1 case per 3 seconds. This case is expected to increase every year, including in Indonesia. In 2050, 1/3 of Indonesia's population is at risk of experiencing osteoporotic fracturesdue to the growth of the 50-70 year age group which is estimated to be 135%(Kemenkes, 2020). This osteoporoticfracture will cause limited ambulation, chronic pain, loss of independence, even reduce the quality of life, and increase the risk of death (Tai et al., 2021)

However, not only osteoporoticfractures that cause a decrease in the quality of life of menopausal women, but osteoporosis in the jawbone that triggers tooth loss causes the same thing as well (Pavlesen et al., 2016). This tooth loss will cause masticatory dysfunction which can affect the decrease in nutritional intake (Kusumawardani et al., 2021). Decreased nutrition due to tooth loss will eventually trigger malnutrition for the elderly,weakness, and ultimately susceptible todisease and death.

Treating osteoporosis in menopausal women isa very important matter. Not only treating the effects caused by osteoporosis, but also by determining how to balance the bones, at least reducing resorption activity while increasing bone apposition. This treatment must be able to regulate the estrogen hormone which is closely related to osteoporosis in menopausal women, as well as calcium metabolism. So far, the therapy used for treating osteoporosis is hormone replacement therapy and bisphosphonates. Hormone (estrogen) replacement (HRT) is commonly used to treat osteoporosis in perimenopausewomen by increasing the estrogen hormone. However, long-term estrogen therapy increases the risk of breast and endometrial cancer, stroke, coronary atherosclerosis, pulmonary embolism, and deep vein thrombosis. Likewise, monoclonal antibody therapy against sclerostin causes various types of adverse reactions or even endangers the patient's life. (Ana-Maria et al., 2021), Meanwhile, bisphosphonates are osteoporosis therapy which is done by increasing bone mineral density and reducing the risk of fractures. Bisphosphonates, which are inorganic pyrophosphate analogs, are

found in bone over a long period of time and are released in very small amounts during bone remodeling. Bisphosphonates work by inhibiting bone resorption activity. However, bisphosphonates can trigger osteonecrosis of the jawbone (Chamizo Carmona et al., 2013).

Currently, high nutritional drinks are being developed to improve the quality of life of the elderly, including menopausal women. One of which is green bean extract. Green beans are a relatively inexpensive source of protein and carbohydrates. These green beans have a lot of mineral content, especially calcium and flavonoids as well as isoflavones(Ganesan&Xu, 2018). Calcium is an essential mineral that is important for bones (Cho et al., 2012). Meanwhile, isoflavones are phytoestrogens that have antioxidant activity and can be crossed with estradiol receptors, so this activity is good for osteoporosis in menopausal women(Lagari & Levis, 2014). This green bean extract drink is expected to be not only as a phytoestrogen but also as an increase in

calcium intake, considering that osteoporosis is not only caused by estrogen deficiency but also lack of calcium intake. In that case, a study on the benefits of green beans on mandibular bone calcium levels and serumin menopausal model animals suspected of having osteoporosis is conducted.

Method of The Research

This research is a laboratory experimental research with Post Test Only Control Group Design. This study used experimental animals, which are 25 female Wistar rats aged 12-16 weeks with a weight of 300-350 grams. Experimental animals were divided into 5 groups: the group without ovariectomy and not receiving greenbean extract (C1); the group without ovariectomy and given intake of green bean extract (C2); the ovariectomy group without green bean juice intake (T1); the ovariectomy group and given green bean juice intake (T2); and the ovariectomy groupand given bisphosphonate therapy (T3).

Before the experimental animals were given treatment, all of them were adapted to the laboratory environment by paying attention to humidity and lighting.Experimental animals were fed and watered ad libitum. After 2 weeks of adaptation, the treatment group underwent ovariectomy as a

mimicking of menopausal conditions which were expected to have an impact on bone.

Ovariectomy is performed bybilaterally removing the ovaries using thedorsal (back) surgical method to minimize injury and speed up the healing process. Before surgery and ovarian retrieval, experimental animals were anesthetized using ketamine/xyzaline at a dose of 80/10 mg/kgBW intramuscularly. The incision was made bilaterally (0.5-1 cm) using a scalpel blade no.11. The ovaries were retracted from the surrounding fat with a silk ligature thread and then were cut using surgical scissors. The wound was sutured using a sterile cut gutand was sprinkled with powdered antibiotics (Dharmayanti et al., 2019). Experimental animals were evaluated daily to observe wounds. After 30 days after ovariectomy, experimental animals in the treatment group were given green bean extract in their diet. After 20 days, bone loss was observed in ovariectomized mice. (Yeyen et al., 2016)

The Making of Green Bean Extract

Green beans are selected by looking at the seeds that are intact and good. Green beans whose seeds are cracked, wrinkled, and moldy were not used because they affect the taste. In addition, gravel, green bean

shells, and dirt must also be separated. Then green beans (1kg) were soaked in 3 liters of water for 4 hours. After being soaked, the green beans were drained and cleaned with water until clean and then boiled until softso that they are easy to pound. Green beans that have been cleaned and pounded then mixed 18 liters of warm water and filtered until separated from the waste.

Green bean extract was given based on the conversion dose of milk needs per day, which is 2 times a day each as much as

200 mL. This intake was converted to experimental animals using a dose conversion from humans (with a bodyweight of 70 kg) to mouse (with a bodyweight of 200 g) which was 0.018 times the dose in humans. The conversion results obtained

0.87 mL/gram BW. In the treatment group, green bean extract was given after 30 days for 1 week.

On the 8th day, all experimental animals were euthanized using a ketamine overdose. Blood was taken intracardially and the mandible was taken. In the examination of blood calcium levels,intracardial blood is inserted into a tube that already contains ethylene diaminetetraacetic

/ EDTA. A total of 0.1 ml of the serumsample was added with 5000 µg/ml potassium until the volume of the solution became 5 ml. The solution was then pipetted and sprayed onto an atomic

absorption spectrophotometer (AAS) to read the concentration of the sample. Calcium levels were obtained by entering the absorbance of the sample in the standard linear regression equation obtained from the standard calcium data. Calcium standard solutions standard used is 200 ppm and the blank uses distilled water. (Tangalayuk et al., 2015)

Data were analyzed by Kruskall- Walls test, Mann Whitney U, and Pearson correlation test with significance ($p<0.05$).

Results

Table 1. blood and bone calcium levels of experimental animals were made with concentrations of 0, 1, 2, 3 and 4 µg/ml Ca. (Yeyen et al., 2016)

Variable	**Kelompok**	**N**	**Calcium Levels**	**P value**
The mandible bone is cleaned and cut. One gram of mandibular bone sample was added with five ml of concentrated nitric acid and left for one hour at room temperature. Then, it was heated on a hot plate at a low temperature for 4-6 hours and also left for one night. Next, 0.4 ml of concentrated sulphuric acid was added and heated on a hot plate for one hour. Add 2-3 drops of a mixed solution of HClO4:HNO3 (2:1) until the color changes to light yellow.				
Blood Calcium				
Mandibular calcium				
Baseline (C1)		5		
w/o ovx with				

	5				
green bean (C2)					
ovx w/o green	5				
bean (T1)					
ovx with green	5				
bean (T2)					
ovx with BSP	5				
(T3)					
Baseline (C1)	5				
w/o ovx with	5				
green bean (C2)		9.28 ±0.49b,c,d	9.67 ±0.59b,c,d	14.76 ±3.96a,b	12.02 ±0.84a,b
		11.53 ±0.72a,b	14.44 ±4.92b,c	13.23 ±1.66b,c,	

0.001

*

_____ 0.001

The sample was transferred and cooled then added two ml of distilled water and 0.6 ml of HCl. Then, it was reheated for 15 minutes and filtered with glass wool into a 100 ml volumetric flask. The ash results were analyzed using atomic absorbance

ovx w/o green

5

bean (T1)

ovx with green

5

bean (T2)

ovx with BSP

5

(T3)

9.61

*

±0.44a,b,d

10.55

±0.34a,b,d11.54

±0.53c,d

spectrophotometric (AAS). The calcium

Calcium is an important mineral inthe body and is the most abundant in the body. This study shows that good calcium levels in the blood are influenced by calcium intake and menopausal conditions. The highest blood calcium level is in the experimental animal group that undergoesovariectomy and without green beans among all treatment groups (14.76 ± 3.96). However, calcium levels in this group were not significantly different from the other treatment groups ($p>0.05$), only significantly different from the control group ($p<0.05$). Likewise, there was no significant difference between the control groups in the average serum calcium level ($p>0.05$).

In contrast to serum calcium levels, the control group has higher bone calcium levels than the treatment group. However, the control group and the treatment group (T3) have the same bone calcium levels ($p>0.05$). The control group (C) and the treatment group (T3) have significant differences in bone calcium levels in the treatment group (T1 and T2) ($p<0.05$). The ovariectomy group without green beans (T1) has a significant difference from the ovariectomized group given green beans (T2).

Table 2. Pearson correlation coefficient of serum calcium and bone calcium levels in animal models

	Serum calcium		Bone calcium	
	r	P value	r	P value
Groups	0.376[b]	0.064	0.438[a]	0.029*
Serum calcium			-0.437[a]	0.029*

Table 2 shows the relationship between serum calcium, bone, and treatment groups. The treatment group, the condition without ovariectomy, ovariectomy, and the given green bean extract. The results show that thetreatment has a weak relationship withserum calcium levels ($r=0.376$; $p=0.064$), but has a strong correlation with bone calcium ($r=0.438$; $p=0.029$). Bone calcium has a negative correlation with serum calcium levels; the higher the serum calcium level, the lower the bone calcium ($r=-0.437$; $p=0.029$).

Discussion

Calcium is an important mineral for bone growth and mineralization by regulating bone turnover and bone mass. In addition, calcium is needed to regulate muscle contraction and relaxation, is involved in nerve transmission, helps blood clotting, and regulates hormones in the body and growth factors (Peacock, 2010). The results of this study indicated that blood calcium levels in the treatment group were higher than in the control group. In

addition, there was no significant difference between the treatment groups. This also indicated that the ovariectomy condition caused the release of calcium in the serum. The ovariectomy condition in the treatment group was thought to trigger bone resorption, which was characterized by an increase in serum calcium levels. Ovariectomy as a mimicking of menopausal conditions would trigger high bone turnover where the osteoclastogenic activity was higher than the osteoblast activity. This caused the release of calcium in the blood or an increase in calcium levels (Kalavathy et al., 2020; Limawan et al., 2015).

In addition, the administration of green bean extract and bisphosphonates within 7 days might not be able to stabilize serum calcium levels. This was probably caused by the amount of calcium in green bean extract and its administration was not accompanied by the administration of vitamin D. The bone remodeling process requires calcium 300-500 mg and calcium from the diet which ranged from 1000 to 1500 mg (Parinduri et al., 2017), so serum calcium was in a homeostatic (balanced) state, while the calcium in green bean extract was only around 80-135 mg (Hou et al., 2019). In addition, calcium absorption in the duodenum requires active vitamin D and protein because the calcium absorption process is active diffusion that requires calcium-binding protein (CaBP) or calbindin. Vitamin D is very important for calcium metabolism because vitamin D controls the absorption, excretion, and mobilization of calcium in the blood, resulting in serum calcium homeostasis (Peacock, 2010; Prasetya et al., 2015; Setyorini et al., 2016).

The results also showed that the control group had higher mandibular bone calcium levels than the treatment group. This indicated that in the treatment group bone resorption was likely to occur. However, bisphosphonates were able to reduce the process of mandibular bone resorption (Arlt et al., 2020; Ezekiel et al., 2017), which in this group had the same bone calcium levels as the control group. Although the calcium content contained in green bean extract was small, green bean extract might be able to increase bone calcium levels and reduce bone resorption (Fu et al., 2014; Ganesan&Xu, 2018; Yeyenet al., 2016). This was proved by the

significant difference between the ovariectomized group with and without green bean extract.

Low calcium intake and low estrogendeficiency can be risk factors in the development of osteoporosis. Supplementation with vitamin D and calcium slightly increase bone mineral density. Calcitriol, the active form of vitamin D, circulates as a hormone in the blood, regulating the concentration of calcium and phosphate in the bloodstream and promoting healthy bone growth and remodeling. (Cho et al., 2012; Kim et al., 2012; Qin et al., 2021)

Ninety-nine percent of extracellular calcium is present in bone in the form of hydroxyapatite, which reflects the balance between the processes of bone formation and resorption. The process of bone resorption by osteoclasts will cause the release of calcium stored in bone into the systemic circulation. This happens toregulate the balance of calcium in the body. Likewise, in the process of bone formation, there will be an improvement in serum calcium levels to become levels that remain within normal limits. While bone resorption will not improve serum calcium within normal limits, serum calcium levels will continue to increase (Cho et al., 2012; Kalavathy et al., 2020; Sakoh et al., 2019).

The increaseof blood calciumconcentration due to bone resorption will stimulate parafollicular cells of the thyroid gland to secrete calcitonin into the blood. At the same time, the parathyroid glands reduce the secretion of parathyroid hormone into the blood. High levels of calcitonin in the blood stimulate the bones to remove calcium from the blood plasma and store it as bone. In addition, the release of calcium from bones is also triggered by blood calcium levels that are too low, resulting in inhibition of calcitonin secretion and stimulation of PTH secretion. This process is carried out in a balanced manner to maintain blood calcium levels (Ana-Maria et al., 2021; Asfur et al., 2018; Thent et al., 2018).

Low calcium levels can be corrected by giving calcium intake. The body's main sources of calcium from the diet include milk and its products, green vegetables, fish, nuts, and calcium-fortified processed foods such as juices and cereals. Green beans contain nutrients, including amylum, protein, iron, sulfur, calcium, fatty oils, manganese, magnesium, niacin, vitamins. Green beans are also rich in minerals such aspotassium, phosphorus, manganese, calcium,

magnesium, iron, zinc, selenium. Calcium ingreen beans is an external intake of calcium in serum which is expected to be depositedin bones (Asfur et al., 2018; Ganesan&Xu, 2018; Prasetya et al., 2015).

In addition, green beans also contain isoflavones that play a role in the process of bone formation. These isoflavones haveactivities like estrogen; they are known as phytoestrogens. Phytoestrogens that can function like estrogen for women can increase bone density and structure, and prevent bone fragility (osteoporosis),especially for women who are in menopause. In addition, it can also reduce postmenopausal syndrome (Ganesan & Xu, 2018; Kim et al., 2012; Yu et al., 2015).

An ovariectomy is an act of removing the female reproductive organs, causing post-menopausal conditions. In post-menopause, there will be a decrease in estrogen production which triggers osteoporosis. Calcium and Isoflavones stimulate osteoblastic activity (formation of bone cells) through the activity of

estrogen receptors and increase the production of growth hormone: insulin-like growth factor -1 (IGF-1) thereby helping bone formation. Isoflavones have an osteoprotective

function, which can prevent osteoporosis orbone loss (Zheng et al., 2016)

Conclusion

Green bean extract was able to reduce serumcalcium levels and increase mandibular bonecalcium levels in experimental menopausal models suspected of having osteoporosis. Further research on the effectiveness of green bean extract in the bone remodeling process with various bone remodeling indicators is still being carried out so that green bean extract can be used as the main diet of postmenopausal women without side effects.

References

Ana-Maria, P., Costinela, G., Claudia, S. S.,Oana, D., & Gabriela, B. (2021).
Treatment strategies in osteoporosis -An analysis of practice guidelines.
Progress in Nutrition, 23(2). https://doi.org/10.23751/pn.v23i2.9748

Arlt, H., Mullarkey, T., Hu, D., Baron, R.,

Ominsky, M. S., Mitlak, B., Lanske, B., & Besschetnova, T. (2020). Effects of abaloparatide and teriparatide on bone resorption and bone formation in female mice. Bone Reports, 13(June), 100291.
https://doi.org/10.1016/j.bonr.2020.100

291

Asfur, R., Tanjung, M. T., & Sari, M. I. (2018). Pengaruh Suplemen Kalsium terhadap Kadar Kalsium Darah dan Bone Mineral Density (BMD) pada Wanita Pengguna Kontrasepsi Depo Medroksi Progesteron Asetat (DMPA). Jurnal Ilmiah Maksitek, 3(4), 145–160. https://makarioz.sciencemakarioz.org/index.php/JIM/article/view/83/80

Chamizo Carmona, E., Gallego Flores, A., Loza Santamaría, E., Herrero Olea, A., & Rosario Lozano, M. P. (2013).
Systematic Literature Review of Biphosphonates and Osteonecrosis of the Jaw in Patients With Osteoporosis.Reumatología Clínica (English Edition), 9(3), 172–177.
https://doi.org/10.1016/j.reumae.2012.07.011

Cho, J. H., Cho, D. C., Yu, S. H., Jeon, Y.

H., Sung, J. K., & Kim, K. T. (2012).

Effect of dietary calcium on spinal bone fusion in an ovariectomized rat model. Journal of Korean Neurosurgical Society, 52(4), 281–287.https://doi.org/10.3340/jkns.2012.52.4.281

Dharmayanti, A. W. S., Hamzah, Z., Meilawaty, Z., Novita, M., Ermawati,

T., Febrianto, B., Putradjaka, A. M., & Cahyani, I. (2019). Ovarian Dysfunction Induced Porphyromonas gingivalis Infection Enhances the Risk of Metabolic Syndrome (in vivo study). Malaysian Journal of Medicine and Health Sciences, 15(suppl 7), 45.

Ezekiel, G., Soundararajan, S., & Tan, C. N. (2017). Spine rehabilitation exercise as an adjuvant to biphosphonates in the treatment of osteoporosis. Osteoporosis and Sarcopenia, 3(3), S30. https://doi.org/10.1016/j.afos.2017.08.054

Fu, S. wen, Zeng, G. feng, Zong, S. hui, Zhang, Z. yong, Zou, B., Fang, Y., Lu,

L., & Xiao, D. qiang. (2014). Systematic review and meta-analysis ofthe bone protective effect of phytoestrogens on osteoporosis in ovariectomized rats. Nutrition Research, 34(6), 467–477. https://doi.org/10.1016/j.nutres.2014.05

.003

Ganesan, K., & Xu, B. (2018). A critical review on phytochemical profile and health promoting effects of mung bean (Vigna radiata). Food Science and Human Wellness, 7(1), 11–33. https://doi.org/10.1016/j.fshw.2017.11.

002

Hou, D., Yousaf, L., Xue, Y., Hu, J., Wu, J.,

Hu, X., Feng, N., & Shen, Q. (2019).

Mung bean (Vigna radiata L.): Bioactive polyphenols, polysaccharides, peptides, and healthbenefits. Nutrients, 11(6), 1–28. https://doi.org/10.3390/nu11061238

Jahan, F. (2015). Osteoporosis : A Silent Killer Women ' s Health Care Osteoporosis : A Silent Killer. J Women's Health Care, 4(January 2015), 4–5.

https://doi.org/10.4172/2167-0420.

Kalavathy, N., Anantharaj, N., Sharma, A., & Chauhan, T. (2020). Effect of serum vitamin D, calcium, and phosphorus on mandibular residual ridge resorption in completely edentulous participants: A clinical study. Journal of Prosthetic Dentistry, 1–7. https://doi.org/10.1016/j.prosdent.2020.07.019

Kemenkes. (2020). Infodatin-Osteoporosis-2020.pdf.

Kim, J.-M., Jin, N., & Park, Y.-S. (2012).

Effects of Legumes Consumption onthe Association of Cholesterol and Bone Mineral Density in Ovariectomized Rats. Korean Journalof Medicinal Crop Science, 20(1), 42–46.

https://doi.org/10.7783/kjmcs.2012.20.1.042

Kusumawardani, B., Wulan, A., Dharmayanti, S., Christmarini, D. M., Rahayu, Y. C., & Fitriyana, N. I. (2021). Correlation between general health toward nutritional and oral health status , and nutrition toward oralhealth status of elderly in a typical retirement community-house. Padjadjaran Journal of Dentistry,33(2), 146–154. https://doi.org/10.24198/pjd.vol33no2.18950

Lagari, V. S., & Levis, S. (2014).

Phytoestrogens for menopausal bone loss and climacteric symptoms. Journal of Steroid Biochemistry and Molecular Biology, 139, 294–301. https://doi.org/10.1016/j.jsbmb.2012.12.002

Limawan, D., Mewo, Y. M., & Kaligis, S.

H. M. (2015). Gambaran Kadar Kalsium Serum Pada Usia 60-74 Tahun. Jurnal E-Biomedik, 3(1). https://doi.org/10.35790/ebm.3.1.2015.6731

Parinduri, F. K., Rahfiludin, M. Z., & P, S.

F. (2017). Hubungan Asupan Kalsium,Vitamin D, Fosfor, Kafein, Aktivitas Fisik Dengan Kepadatan Tulang Pada Wanita Dewasa Muda (Studi Kasus Pada Mahasiswi S1 Reguler Fakultas Kesehatan Masyarakat Universitas Diponegoro Angkatan 2014). Jurnal Kesehatan Masyarakat (e-Journal), 5(4), 664–674.

Pavlesen, S., Mai, X., Wende, J. W., LaMonte, M. J., Hovey, K. M., Genco,

R. J., & Millen, A. E. (2016). Vitamin D status and prevalent and incident tooth loss in postmenopausal women: The Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) Study. J Periodontol, 87(8), 852–863. https://doi.org/10.1902/jop.2016.150733.Vitamin

Peacock, M. (2010). Calcium metabolism in health and disease. Clinical Journal of the American Society of Nephrology, 5(SUPPL. 1), 23–30. https://doi.org/10.2215/CJN.05910809

Prasetya, D., Wirjatmadi, B., & Adriani, M. (2015). Pengaruh Pemberian Susu yang Difortifikasi (Kalsium dan Vitamin D) dan Senam Osteoporosis terhadap Kepadatan Tulang pada Wanita Pra Lansia di Wilayah Kerja Puskesmas Banyuanyar Kabupaten Sampang. Jurnal Ilmiah Kedokteran, 4(1), 25–38.

Qin, X. yan, Niu, Z. chang, Han, X. ling, Yang, Y., Wei, Q., Gao, X. xue, An, R., Han, L. feng, Yang, W. zhi, Chai, L. juan, Liu, E. wei, Gao, X. mei, & Mao, H. ping. (2021). Anti-perimenopausal osteoporosis effects of Erzhi formula via regulation of bone resorption through osteoclast differentiation: A network pharmacology-integrated experimental study. Journal of Ethnopharmacology, 270(November 2020), 113815. https://doi.org/10.1016/j.jep.2021.113815

Sakoh, T., Taniguchi, M., Yamada, S., Ohnaka, S., Arase, H., Tokumoto, M., Yanagida, T., Mitsuiki, K., Hirakata, H., Nakano, T., Kitazono, T., & Tsuruya, K. (2019). Short- and Long- term Effects of Dialysate Calcium Concentrations on Mineral and Bone Metabolism in Hemodialysis Patients:The K4 Study. Kidney Medicine, 1(5),296–306. https://doi.org/10.1016/j.xkme.2019.08.002

Setyorini, A., Suandi, I., Sidiartha, I. G. L., & Suryawan, W. B. (2016). Pencegahan Osteoporosis dengan Suplementasi Kalsium dan Vitamin D pada Penggunaan Kortikosteroid Jangka Panjang. Sari Pediatri, 11(1), 32. https://doi.org/10.14238/sp11.1.2009.32-8

Szamatowicz, M. (2016). How can gynaecologists cope with the silent killer - Osteoporosis? Przeglad Menopauzalny, 15(4), 189–192. https://doi.org/10.5114/pm.2016.65682

Tai, T.-W., Li, C.-C., Huang, C.-F., Chan,

W. P., & Wu, C.-H. (2022). Treatment of osteoporosis after hip fracture is associated with lower all-cause mortality: A nationwide population study. Bone, 154(May 2021), 116216. https://doi.org/10.1016/j.bone.2021.116216

Tangalayuk, reggy raisa, Suarsana, i nyoman, & iwan harjono utama. (2015). Kadar Kalsium dan Fosfor Pada Tulang Tikus Betina yang Diberi Tepung Tempe Rendah Lemak. BuletinVeteriner Udayana, 7(1), 60.

Thent, Z. C., Froemming, G. R. A., Ismail,

A. B. M., Fuad, S. B. S. A., & Muid, S.(2018). Employing different types of

phytoestrogens improve bone mineralization in bisphenol A stimulated osteoblast. Life Sciences,210(July), 214–223.
https://doi.org/10.1016/j.lfs.2018.08.057

Yeyen, A., Bahtiar, A., & Soekanto, S. (2016). Pengaruh Pemberian Sari Kacang Hijau (Vigna radiata (L .) R .Wilczek) Terhadap Densitas Tulang dan Jumlah Osteoklas. Jurnal FarmasiIndonesia, 8(2).

Yu, F., Liu, Z., Tong, Z., Zhao, Z., & Liang,

H. (2015). Soybean isoflavone treatment induces osteoblast differentiation and proliferation by regulating analysis of Wnt/β-catenin pathway. Gene, 573(2), 273–277. https://doi.org/10.1016/j.gene.2015.07.054

Zheng, X., Lee, S. K., & Chun, O. K. (2016). Soy Isoflavones and Osteoporotic Bone Loss: A Review with an Emphasis on Modulation of Bone Remodeling. Journal of Medicinal Food, 19(1), 1–14. https://doi.org/10.1089/jmf.2015.0045