CAFFEINE MAY BE SAFE FOR WHITE MALE RATS BONE DENSITY

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ABSTRAK

Konsumsi tinggi kafein, diyakini oleh beberapa ilmuwan, dapat menyebabkan osteoporosis yang ditandai dengan penurunan kepadatan mineral tulang dan kerusakan arsitektur mikro tulang. Tujuan dari penelitian ini, adalah untuk membuktikan apakah kafein dapat menurunkan kepadatan mineral tulang yang mengarah ke osteoporosis atau tidak. Penelitian menggunakan desain pretest-posttest sebagai metode, serta sampel untuk penelitian ini adalah 40 Tikus putih Jantan (Rattus norvegicus) yang dibagi menjadi 4 kelompok masing-masing berisi 10 ekor tikus. Satu kelompok digunakan sebagai kelompok kontrol, diberikan 3 ml air untuk masing-masing tikus. Sementara kelompok lain menerima 3 dosis yang berbeda, ada 2,25 mg, 6,75 mg, 11,25 mg dan diberikan setiap hari selama sebulan. Kepadatan tulang diukur dengan DBM Sonic 1200. Analisis data dilakukan dengan menggunakan uji Analisis Varians (Anova). Tingkat signifikansi dalam penelitian ini adalah 0,089. Hasil berarti bahwa tidak ada yang berbeda dari kepadatan mineral tulang antara kelompok kontrol dan kelompok yang telah diberikan dengan kafein. Secara teoritis kafein dapat mengurangi kepadatan tulang tetapi secara statistik tidak ada pengaruh kafein dalam menurunkan kepadatan mineral tulang. ***(FMI 2015;51:74-79)***

Kata kunci: *osteoporosis, kepadatan mineral tulang, kafein*

ABSTRACT

High consumption of caffeine, believed by some scientist, can cause osteoporosis which marked by decrease in bone mineral density and destruction of bone micro architecture. The purpose of this experiment, was to prove whether caffeine could decrease bone mineral density which lead into osteoporosis or not. The experiment used pretest-posttest design as its method, and the sample for this experiment were 40 White Male Rats (Rattus norvegicus) which were separated into 4 groups each contain 10 rats. One group used as control group, given 3 ml of water for each rat. While the other groups received 3 different dose, there were 2.25 mg, 6.75 mg, 11.25 mg and given daily for a month. Bone density was measured with DBM Sonic 1200. Data analysis was done using Analysis of Variance (Anova) test. The level of significance in this experiment was 0.089. The results meant that there were no different of bone mineral density between control group and groups that had been given with caffeine. Theoretically caffeine can reduce bone density but statistically there were no effect of caffeine in decreasing bone mineral density. ***(FMI 2015;51:74-79)***

Keywords: *osteoporosis, bone mineral density, caffeine*

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INTRODUCTION

Decreased bone density is the initial symptom onset of osteoporosis. Osteoporosis is defined as a disease characterized by reduced bone mass and bone micro-architecture followed damage resulting increase in bone fragility and lead to bone fracture (Kusumo 2004). The onset of osteoporosis can be caused by excessive consumption of caffeine. Caffeine or methyl xanthines are compounds that are often found in a wide variety of foods and beverages, such as coffee, tea, carbonated beverages, energy drinks, chocolate, and others. When consumed in excessive doses, caffeine is believed to reduce the level of bone density or bone mineral density.

Above hypothesis is supported by several studies that have been done. In one research journal stated that the consumption of caffeine in high doses may increase the risk of porous bone and lead to the onset of osteoporosis. This can occur due to the increased excretion of calcium in the kidneys, as well as interfere with the bone remodeling process, because an interruption in the working of parathyroid hormone (Nawrot et al 2003). Decreased activity of osteoblast cells can enhance apoptosis of osteoblast so that can cause a decrease in the rate of bone density (Tsuang et al 2006).

Based on the above theories can be known that, caffeine through a variety of mechanisms, can result in a

decrease in bone density is directly or indirectly (inhibiting hormones that affect bone). This is why research is done to prove whether caffeine can cause porous bone and seek at how many doses of caffeine can lead to decreased bone density.

MATERIALS AND METHODS

This research is an experimental study with the analytical approach the pretest-posttest design. Measurement of bone density was performed before the experimental animals were given caffeine (pretest) and after being given caffeine (posttest). The experiment was conducted in a couple of stages, the first measurement of bone density throughout the rat was held on October 7, 2011 before the treatment is given in the form of addition of caffeine. Then proceed with the granting caffeine which began on October 21, 2011 to 21 November 2011. Bone density measurements performed 5 times during the study, measurements were performed 1 time per week, in the laboratory the Department of Pharmacology Faculty of Medicine Universitas Airlangga. The sample used in this study were white male rats (*Rattus norvegicus*) with its main criteria, Must be 2 to 2.5 months old with a weight of 200 grams, Male and Healthy (marked by an active movement). The number of samples was 10 white male rats were taken from each group, which in this research, there are 4 groups, so that the total sample is 40 head.

How to capture data using a data collection method of bone density of white rats performed one time each week both before treatment and after is given treatment and measured with a DBM Sonic. Bone density measured at the femoral metaphysis. This measurement needs to performed anesthetic on white rats with ether and epilation in rats. Measurements were performed four times and the average results are shown in the monitor recorded.

Bone density in male white rats of each group will be compared with the bone density of white rats in group A (rats by administration of 3 ml of distilled water/200 g body weight/day). These groups are, group B (2.25 mg of caffeine in 3 ml of distilled water/200 g bw/day), group C (6.75 mg of caffeine in 3 ml of distilled water/200 g bw/day), group D (11, 25 mg of caffeine in 3 ml of distilled water/200 g body weight/day). Density measurements of the femur metaphysis bone of experimental animals was measured by DBM Sonic 1200, with provisions the sooner or high value (m/sec), the greater the bone density. The data have been collected and processed was described with SPSS 11.5 for windows. Then the data control group and the treatment group were tested statistically by ANOVA test (analysis of variance). ANOVA tests were performed by the method of one-way ANOVA/one way, because it is only comparing one parameter only, ie how many levels can lead to decreased bone density.

RESULTS

Before analyzing the data using ANOVA test, then the data to be tested must meet several requirements in advance, ie the data must be normally distributed and homogeneity of variance should be normal. To determine the normality of the data used the Kolmogorov-Smirnov and Shapiro-Wilk. While the homogeneity of variance using the homogeneity test. If the data meets the requirements will be followed by ANOVA test and advanced test Post Hoc Multiple Comparison.

Normality test data

Kolmogorov-Smirnov normality test aims to determine whether the measurement data is normally distributed or not. In normally distributed data, the test will be obtained significance value (p) ≥ 0.05 for all data. If the data is normally distributed, it can proceed with the ANOVA test, if the data are not normally distributed with the ANOVA test should not be used.

Addition to using the Kolmogorov-Smirnov and Shapiro Wilk, normal distribution of the results of this study can be seen from Chart distribution of bone density.

Table 1. Normality test data

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Control	.163	10	.200*	.958	10	.767
Caffeine 2.25 mg	.130	10	.200*	.979	10	.961
Caffeine 6.75 mg	.212	10	.200*	.892	10	.180
Caffeine 11.25 mg	.236	10	.120	.909	10	.273

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

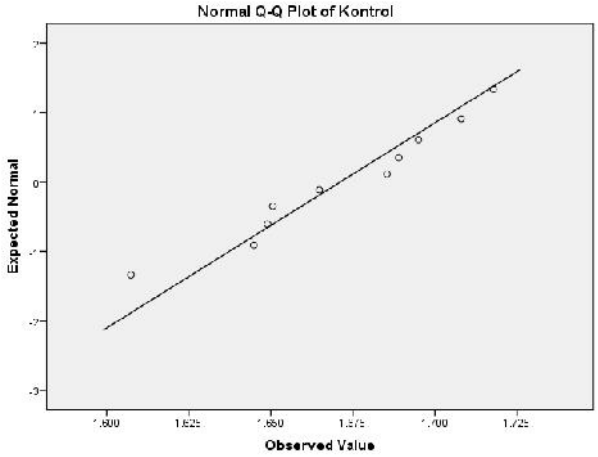

Figure 1. Graph of the distribution of group A

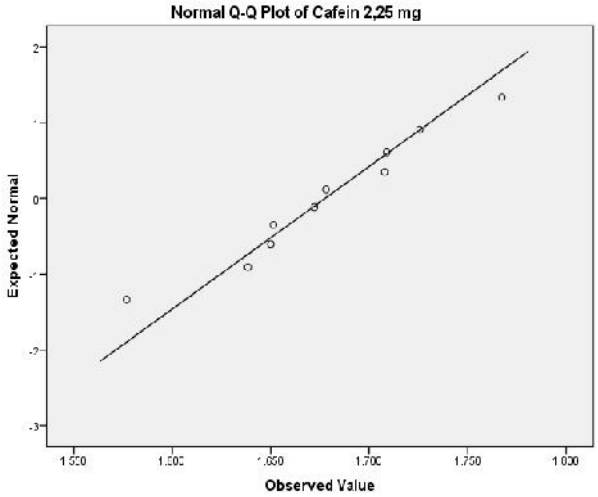

Figure 2. Graph distribution of group B

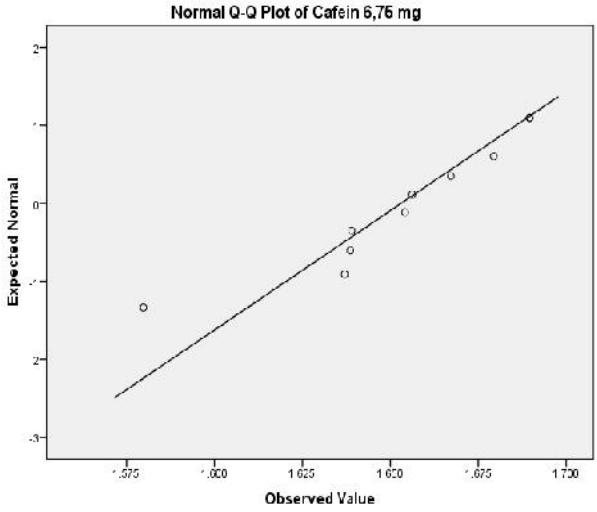

Figure 3. Graph distribution of group C

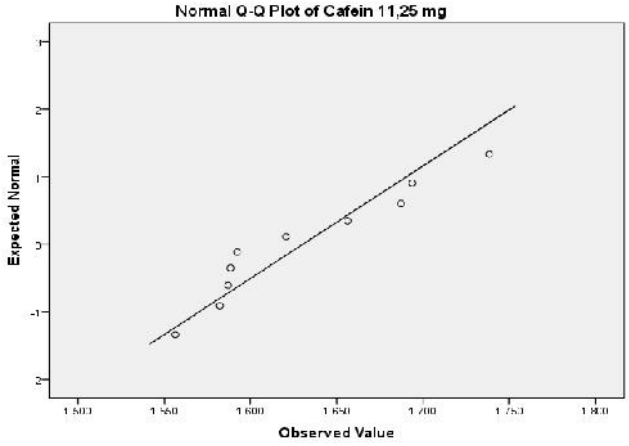

Figure 4. Graph distribution of group D

Because all four data group has $p > 0.05$, it can be concluded that the four groups of normally distributed data (homogeneous).

Homogeneity test data

Requirements to perform another ANOVA test was the normal variance homogeneity test. In the data has a normal homogeneity it will get the value of significance (p) ≥ 0.05, the data obtained in this study have a value of 0.180 homogeneity. The data of this study are eligible for Anova test.

Table 2. Test of hemogenity of variances Caffeinee

Levene Statistic	df1	df2	Sig.
1.723	3	36	,180

ANOVA test

ANOVA (Analysis of Variance) Test aimed to compare several groups of samples (more than two groups of samples). ANOVA test can be done if the data were normally distributed and variants derived from homogeneous.

Table 3. Anova Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19700.883	3	6566.961	2.342	.089
Within Groups	100939.948	36	2803.887		
Total	120640.831	39			

P value in the ANOVA test was 0.089, meaning that there is likely no significant difference in the four groups of data. To determine the existence of different groups then we carried out multiple comparison test. The multiple comparison test revealed no differences between groups of data. Because the p value are all > 0.05, based on these results, it can be concluded that there was no difference in bone density between the groups of rats were given caffeine in various doses to the control group (Table 4). Table 5 is a table of the average bone density test animals were measured during the study. Results of animal bone density contained in the below table were averaged each week. The average yield is used to determine the dose-response test. Table 6 is a table of dose response. In this table, the figures show the percent decrease in bone density of rats, the rate is obtained by the formula (x-x1)/x×100 %. Where X is the average of the control group and X1 is the average of the treated group (A, B, and C). The calculation of dose response starts from week to week II to V, since the first week of experimental animals has not given caffeine. In the dose-response trial found a decrease in the level of increase in bone density in Week II, III, and IV along with the large dose of caffeine given to the experimental animals. In the weeks to V, we did not obtain more bone density decrease at higher doses.

Table 4. Multiple comparisons test

			Mean Difference			95% Confidence Interval	
	(I) Control	(J) Control	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
LSD	Placebo	Caffeine 2.25 mg	-6.46000	23.68074	.787	-54.4868	41.5668
		Caffeine 6.75 mg	40.92000	23.68074	.093	-7.1068	88.9468
		Caffeine 11.25 mg	40.92000	23.68074	.093	-7.1068	88.9468
	Caffeine 2.25 mg	Placebo	6.46000	23.68074	.787	-41.5668	54.4868
		Caffeine 6.75 mg	47.38000	23.68074	.053	-.6468	95.4068
		Caffeine 11.25 mg	47.38000	23.68074	.053	-.6468	95.4068
	Caffeine 6.75 mg	Placebo	-40.92000	23.68074	.093	-88.9468	7.1068
		Caffeine 2.25 mg	-47.38000	23.68074	.053	-95.4068	.6468
		Caffeine 11.25 mg	.00000	23.68074	1.000	-48.0268	48.0268
	Caffeine 11.25 mg	Placebo	-40.92000	23.68074	0.93	-88.9468	7.1068
		Caffeine 2.25 mg	-47.38000	23.68074	.053	-95.4068	.6468
		Caffeine 6.75 mg	0.00000	23.68074	1.000	-48.0268	48.0268

Table 5. Mean and standard deviation levels of bone density in rats per group

Group	Weeks					Mean ± SD		
	Week I	Week II	Week III	Week IV	Week V			
A	1584	1639	1720	1699	1716	1671.6	±	58.7
B	1626	1623	1699	1685	1745	1675.6	±	51.7
C	1633	1639	1594	1700	1685	1650.2	±	42.6
D	1646	1570	1581	1668	1705	1634	±	57.5

Table 6. Dose response

Group	Week I	Week II	Week III	Week IV
B	1	1	1	0
C	0	7	0	2
D	4	8	2	1

In the dose-response trial found a decrease in the level of increase in bone density in Week II, III, and IV along with the large dose of caffeine given to the experimental animals. In weeks V, we did not obtain increase in bone density decrease at higher doses.

DISCUSSIONS

Research on the effect of caffeine on bone density white male rats aimed to prove that caffeine may decrease bone density and may lead to the onset of osteoporosis. Caffeine itself is a chemical compound that is widely available in foods and beverages that are consumed every day, especially in coffee. Doses used in this study were 2.25 mg, 6.75 mg and 11.25 mg. This dose levels obtained from caffeine found in one cup of coffee and converted into the appropriate dose for rats. Dose of 2.25 mg is equal to 1 cup of coffee; 6.75 mg is equal to 3 cups of coffee and a dose of 11.25 mg equal to 5 cups of coffee. Rats used were white rats (*Rattus norvegicus* Wistar strain) with 2-2.5 months of age with a body weight of 200 grams. Rat tails were used by 40 and divided into 4 groups, each consisting of 10 rats. Each

treatment group was given different doses of caffeine, while the control group was given distilled water only. Caffeine is entered first dissolved into distilled water and 3 cc intragastric for 30 days. Bone density was measured every week using bone densitometry DBM Sonic 1200.

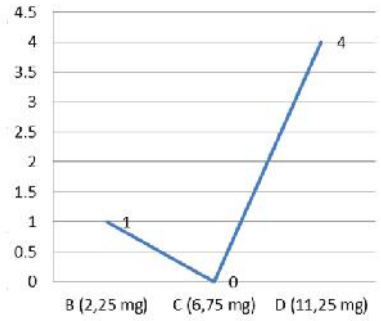

Figure 5. Dose response curve in the second week

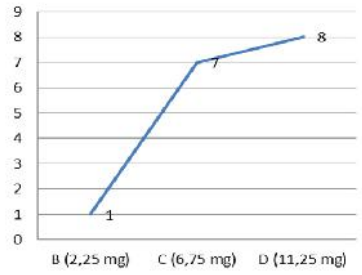

Figure 6. Dose response curve in the third week

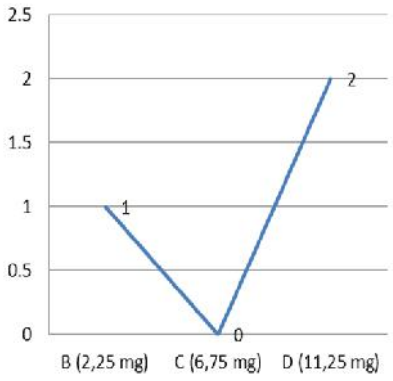

Figure 7. Dose response curve in the fourth week

In theory, the more caffeine consumed, then a bone density will decrease because caffeine can inhibit the action of parathyroid hormone to increase calcium excretion and also interfere with the process of bone remodeling. Besides caffeine also caused a decline in work cells and osteoblasts in bone cells also increased the apoptosis of osteoblasts. These things can lead to a decreased level of bone density.

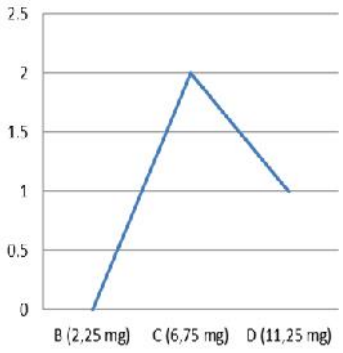

Figure 8. Dose response curve in the fifth week

However, research conducted in the Laboratory of Pharmacology, Faculty of Medicine, Universitas Airlangga, for 31 days, did not show results that indicate that high doses of caffeine can cause a decrease in bone density. It can be seen from the average speed of bone densitometry DBM Sonic 1200 in the control group and the treatment group showed no significant difference.

To prove further, research data obtained were tested using ANOVA test. The results showed that there was no difference in bone density levels between group of rats were given caffeine in various doses to the control group $p = 0.089$. It can be concluded that there is no statistically significant difference between the levels of bone density rats control group to the treatment group in various doses.

Unremarkable effect of caffeine in this study may be caused by (1) Shorter time of study. On the effects of caffeine Nawrot et al (2003) stated that the consumption of high doses of caffeine on a regular basis every day can interfere with the process of bone remodeling in approximately 19 days. In this study, experiments were conducted for 31 days, based on the possibility of the journal has been established in the bone remodeling process disruption but not cause a decrease in bone density in experimental animals. (2) The dose given less appropriate, because the doses used in this study is a dose equivalent to one cup of coffee (2.25 mg), three cups of coffee (6.75 mg) and five cups of coffee (11.25 mg) then this dose converted to dose administration in rats using a conversion table. The effect of decreasing bone density in rats may be obtained at a higher dose than the dose given in this study. (3) The influence of calcium from the food given to the animal because the effect of a decrease in bone density can be reduced by caffeine when accompanied by calcium administration.

(4) Active movement and sound of animals try to affect the speed of ultrasound bone densitometry. Active movements of animals could increase or decrease the speed of sound conductivity DBM sonic tool while animal sounds can interfere with the transmission of sound from the DBM sonic tool because of the difference in acoustic impedance of the sound of rats and gauges. (5) The tools used in this study (DBM Sonic) is less sensitive for examination using the tool only shows the speed of sound in bone conductivity, does not really show the presence of porous bone.

CONCLUSION

No reduction in the effect of caffeine was found in rats bone density in rats. Further studies to prove the effect of caffeine on bone density decline should (1) be done in a longer period of time to get the effect of a decrease in bone density. (2) not to feed the experimental animals with high doses of calcium-containing, (3) and use more sensitive and more sophisticated bone density gauges.

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