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Supplementation of tempeh extract for improving quality of bone in premenopausal conditions using rats as animal models

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Abstract. Tempeh extract is a natural substance that contains phytoestrogens, having similar estrogen activity. This study was designed to determine the potential of tempeh extract in improving the quality bone in premenopausal conditions, and to compare the natural product of tempeh extract with commercially available hormones (genistein, ethinylestradiol, and somatotropin). Experimental design used was Completely Randomized Design (CRD) consisted of 7 experimental groups, each consisted of 3 rats i.e.,1) K = premenopausal rats as a negative control, 2) P = premenopausal rats given distilled water orally as a placebo, 3) TEM = premenopausal rats given tempeh extract 300 mg/day/200g body weight, 4) GEN = premenopausal rats given genistein 0.25 mg/day/kg body weight, 5) EST = premenopausal rats given ethinylestradiol $9x10^{-3}$ mg/day/200g body weight, 6) SO = premenopausal rats injected with sesame oil/day/kg body weight, 7) BST = premenopausal rats injected somatotropin 9 mg/day/kg body weight. The parameters observed were the bone and serum calcium and phosphorus concentrations, bone ash concentrations, bone collagen concentrations, bone water concentrations, bone RNA concentrations, bone length, bone weight, bone density, and bone strength. Supplementations of tempeh extract for two months in premenopausal rats could improve the quality of bone, which was characterized by the increased bone calcium concentrations, ratio of Ca/P on tibia bone, bone ash levels, bone density, and bone strength.

Key words: Bones ,extract tempeh, rats, premenopausal.

Introduction

Basically, aging is a common physiological process and it takes place continuously marked by changes in the body's cells . The aging occurs because of cells damage , old , and died. Aging mostly associated with the death of the cells (Ganong 2003). Actually, the body has the ability to repair the damaged cells and replace the died cells. However, by increasing of age effect the repair process of cell and turnover is being slow. Accumulations of dead cells inhabit the function of tissues, organs, and physiological functions of the body in general. Human enter premenopausal age period of approximately at 40 years of age, which is characterized by decreasing of ovarian function gradually (Zulkarnaen 2003), and progesterone levels begin to decline (Walker 1995). When increasing of age cause changes in bone quality. At the age between 35-40 years old, bone resorption exceeds bone formation so that bone mass loss estimated at 1% per year (Endris and Rude 1994). Compston et al . (1993) stated that the higher bone mass density at premenopausal period can sustain loss of bone calcium deposits or calcium decline at menopause and age increase and it will avoid body from osteoporosis and fractures at postmenopausal period.

Tempeh contain isoflavone aglycone and glycoside compounds (King 2002), as well as contain nutrients (Directorate of Health Ministry Republic of Indonesia 1995). Tempeh flour contains more isoflavone aglycone compounds if compared with soybean flour (Safrida 2008). Structure of isoflavones can be transformed into equol , and the equol has a phenolic structure similar to the hormone estrogen (Setchell and Cassidy1999) . Use of fresh tempeh requires a large amount to obtain sufficient effects such as the effects of estrogen, so tempeh are processed into tempeh extracts to form the smaller dose and more practical when it consumed.

This study is interesting and to be important because of tempeh extract supplementation as a natural ingredient good to consume at premenopausal and it is also an alternative preventive effort to risk disease when entering postmenopausal age. The purpose of this study is to (1), knowing the potential of Tempeh extract in improving the quality of bone in premenopausal conditions, (2), Comparing natural products from tempeh extract with hormone products are already marketed (genistein, ethinylestradiol, and somatotropin).

Materials and Methods

This study was conducted from May 2011 - April 2012. 15-month-old rats were placed in a plastic cage with a lid made of wire and covered with chaff ram. The feed is the pellets and drinking water are provided ad libitum. Environment was adjusted not to damp , adequate ventilation and adequate lighting for 14 hours and dark 10 hours long. Each rat was placed in individual cages.

The rats were divided into 7 experimental treatment 1) K = premenopausal rats as a negative control, 2) P = premenopausal rats given distilled water orally as a placebo, 3) TEM = premenopausal rats given tempeh extract 300 mg/day/200g body weight, 4) GEN = premenopausal rats given genistein 0.25 mg/day/kg body weight, 5) EST = premenopausal rats given ethinylestradiol $9x10^{-3}$ mg/day/200g body weight, 6) SO = premenopausal rats injected with sesame oil/day/kg body weight, 7) BST = premenopausal rats injected somatotropin 9 mg/day/kg body weight. All groups of rats were given treatment for 2 months. Tempeh extract, genistein and ethinylestradiol given by orally as much as once a day, while a somatotropin injected intramuscularly once a day on the back of the thigh.

At the end of the experiment and the status of the diestrus phase, all rats were sacrificed. Tibia - fibula bones left and right side separated from soft tissue using a small pair of scissors, then left tibia put in BNF 10 % for analysis of collagen concentrations, RNA concentrations, bone density, and bone strength, while the right tibia stored in freezer at - 20 ° C for analysis of calcium concentrations, phosphorus concentrations, and ash concentrations.

Parameters observed are bone collagen concentrations conducted in accordance with the method by Manalu and Sumaryadi (1998), bone ash concentrations (AOAC 1990), bone length, bone weights, bone density (method Arjmandi et al. 1996), and tibia bone

strength test is the adoption of glulam compressive strength test methods conducted by Bahtiar (2008), and compressive strength test timber (Mardikanto et al. 2011).

The experimental design used was a completely randomized design (CRD). The data obtained were analyzed using Analysis of Variance (ANOVA) and followed by Duncan test with 95 % confidence interval (α=0.05), and correlation test using SAS software 9.1.3 software.

Results and Discussion

Effect of supplementation of tempeh extract on bone quality in premenopausal conditions

Mean bone collagen concentrations, bone water concentrations, bone RNA concentrations in premenopausal rat is presented in Table 1. Statistical analysis showed that the tempeh extract, genistein, ethinylestradiol, and somatotropin does not affect the collagen content, water content, and RNA content of bone in premenopausal conditions.

Table 1 Mean bone collagen concentrations, bone water concentrations, bone RNA concentrations in premenopausal rat

Groups	bone collagen concentrations (mg/g sample)	bone water concentrations (%)	bone RNA concentrations (mg/g sample)
K	17.69±0.32	28.67±7.59	10.69±0.78
P	17.31±0.47	28.62±5.07	9.59±1.30
TEM	19.29±1.81	28.16±0.41	11.67±1.57
GEN	19.08±0.64	28.77±5.12	9.98±0.54
EST	19.46±2.01	26.82±3.20	10.86±0.59
SO	16.90±1.41	28.31±1.55	10.23±0.76
BST	19.02±0.61	31.74±2.29	11.17±0.95

Description: K = premenopausal rats as a negative control, P = premenopausal rats given distilled water orally as a placebo, TEM = premenopausal rats given tempeh extract 300 mg/day/200g body weight, GEN = premenopausal rats given genistein 0.25 mg/day/kg body weight, EST = premenopausal rats given ethinylestradiol $9x10^{-3}$ mg/day/200g body weight, SO = premenopausal rats injected with sesame oil/day/kg body weight, BST = premenopausal rats injected somatotropin 9 mg/day/kg body weight.

Tempeh extract, genistein, ethinylestradiol, and somatotropin can increase bone collagen concentrations, but it was not statistically significant. Increased bone collagen causes bones to become stronger and not easily broken. Mean bone calcium and phosphorus concentrations, ratio of Ca/P on tibia bone and bone ash concentrations in premenopausal rats are presented in Table 2. Tempeh extract, genistein, ethinylestradiol, and somatotropin can increase bone calcium and ratio of Ca/P on tibia bone ($P>0.05$) in premenopausal rats. The average bone ash concentrations in premenopausal rats affected ($P > 0.05$) by the extract of tempeh, but not affected by given genistein, ethinylestradiol, and somatotropin .

Table 2 Mean bone calcium and phosphorus concentrations, ratio of Ca/P on tibia bone and bone ash concentrations in premenopausal rats

Group	bone calcium concentrations (%)	bone phosphorus concentrations (%)	ratio of Ca/P on tibia bone (%)	bone ash concentrations (%)
K	32.69 ± 1.94^{cd}	31.10 ± 5.33^{ab}	1.076 ± 0.22^{cd}	31.90 ± 3.86^{b}
P	32.96 ± 2.05^{cd}	29.82 ± 1.52^{b}	1.105 ± 0.05^{cd}	31.04 ± 1.45^{b}
TEM	52.14 ± 2.28^{a}	24.60 ± 1.59^{b}	2.122 ± 0.10^{a}	37.31 ± 1.20^{a}
GEN	46.24 ± 8.12^{ab}	25.11 ± 2.22^{b}	1.861 ± 0.44^{ab}	33.93 ± 2.20^{ab}
EST	41.06 ± 6.54^{bc}	27.38 ± 1.70^{b}	1.510 ± 0.30^{bc}	34.97 ± 4.56^{ab}
SO	30.03 ± 3.82^{d}	29.89 ± 1.84^{b}	1.008 ± 0.16^{d}	30.26 ± 1.83^{b}
BST	43.31 ± 4.47^{b}	36.27 ± 6.03^{a}	1.021 ± 0.16^{cd}	32.60 ± 1.12^{ab}

Values followed by different letters in the same column showed the significantly different ($p<0.05$).

Tempeh extract able to prevent the premenopausal osteopenia. Karaguzel and Holick (2010) reported in premenopausal women found osteopenia if the lack of calcium. Increase in bone calcium cause bone matrix will be dense and brittle bones. Calcium have a large compressive force on the bone structure (Guyton 1996). The ratio of Ca/P on bone in rats given a tempeh extract, genistein, ethinylestradiol, and somatotropin still within the normal range. A bone ash concentration describes the total amount of inorganic minerals found in bone. Tempeh extract showed an increase in bone ash concentrations, in addition to calcium and phosphorus, which means another on bone mineral content also increased. According Djojosoebagio (1996) bone mineral is an inorganic form of bone, hydroxyapatite crystals with the main mix. In addition to calcium and phosphorus, bone also contains citrate, sodium, barium, strontium, tin, carbonate, fluorine, chlorine, magnesium, and potassium.

The average bone length, bone weight, bone density, and bone strength in premenopausal rat presented in Table 3. Statistical analysis showed that bone length in premenopausal rats is not affected by the treatment, whereas the bone weight, bone density, and bone strength in premenopausal rats affected by treatment ($P < 0.05$) (Table 3). The average bone weight in premenopausal rats given tempeh extracts, genestein, ethinylestradiol, and somatotropin similar to the control rats. The results showed that the bone density, and bone strength in premenopausal rats influenced ($P > 0.05$) by the tempeh extract, but not affected by given genistein, ethinylestradiol, and somatotropin .

Table 3 Mean bone length, bone weight, bone density, and bone strength in premenopausal rats

Group	bone length (cm)	bone weight (g)	bone density (g/mL)	bone strength (kg/cm^2)
K	3.86±0.01	$0.44±0.06^{ab}$	$1.35±0.05^{b}$	$54.08±17.35^{b}$
P	3.83±0.03	$0.43±0.01^{ab}$	$1.34±0.19^{b}$	$52.66±14.68^{b}$
TEM	3.85±0.05	$0.51±0.03^{a}$	$1.61±0.13^{a}$	$93.74±2.183^{a}$
GEN	3.81±0.07	$0.46±0.03^{ab}$	$1.46±0.09^{ab}$	$74.37±23.75^{ab}$
EST	3.84±0.11	$0.47±0.003^{ab}$	$1.42±0.13^{ab}$	$72.37±32.56^{ab}$
SO	3.85±0.01	$0.42±0.03^{b}$	$1.33±0.07^{b}$	$56.66±6.723^{b}$
BST	3.84±0.02	$0.49±0.04^{ab}$	$1.48±0.15^{ab}$	$68.80±13.45^{ab}$

Values followed by different letters in the same column showed the significantly different. ($p<0.05$).

Bone density and bone strength in premenopausal rats has a correlation value (0.96) and shows a correlation significantly different ($P<0.01$), which means that the higher the density of bone increases bone strength. This suggests that the increase in bone density followed by an increase in bone strength. Increased bone density in premenopausal rats prevent osteoporosis in postmenopausal age when entering. This is in line with research Compston et al. (1993) which states that the density higher bone mass in premenopausal period can sustain that loss of bone calcium deposits or calcium decline at menopause and age increase will be spared of postmenopausal osteoporosis and fractures .

Increase of bone strength is important for maintaining bone not easily broken. Tempeh extract can increase bone calcium levels were followed by increased bone strength. This is in line with research Faibish et al. (2006) reported that human bone strength increases with mineral content were found. Tempeh extract showed improvement in the condition of premenopausal bone quality, which is characterized by the increased bone

calcium concentrations, ratio of Ca/P on tibia bone, bone ash levels, bone density, and bone strength

Conclusion

Supplementations of tempeh extract for two months in premenopausal rats could improve the quality of bone, which was characterized by the increased bone calcium concentrations, ratio of Ca/P on tibia bone, bone ash concentration, bone density, and bone strength.

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