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Local Non-Soy *Isoflavone* Attenuates Bone Loss in The Menopausal Women

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Non-Soy; *Isoflavone*; Phytoestrogen; Osteoporosis; Bone Mineral Density; Postmenopausal Women

ABSTRACT

Introduction: Identification and isolation of local non-soy *Isoflavone* has been made based on cowpea (*Vigna unguiculata*) by The Indonesian Menopause Society (PERMI) in Malang District, Indonesia. No published studies have directly examined the effect of that non-soy *Isoflavone* on bone mineral density in menopausal women. This study examined the effect of non-soy *Isoflavone* on bone mass density in menopausal women.

Methods: A randomized, double-blind study with 59 menopausal women divided into two groups administered either 67.5 mg/day of *Isoflavones* (n = 30) or placebo (n = 29) for 24 weeks. At baseline and posttreatment, bone mineral density (BMD) was measured by using densitometry. Collected data were analyzed by Analysis of Covariance (ANCOVA).

Results: There were differences in BMD changes between the treatment and placebo groups for 24 weeks of intervention. The mean of BMD changes in treatment group were 0,952 (p-value= 0,006, SE=-0,221) from baseline, and the placebo group had mean at -0,768 (p-value=-0,006, SE=-0,212). Despite this, coffee as the concomitant variable significantly affected BMD changes (p value=0,043) at the 3rd-month evaluation, but not in 6 months. Participant compliance is good.

Conclusion: Non-soy *Isoflavone* attenuates bone loss in menopausal women.

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INTRODUCTION

All healthy women will experience menopause. Women are considered to have reached menopause if they are neither pregnant nor postpartum amenorrhea and have not had a menstrual period in the 6 months before the survey, or if they report being menopausal, or have never menstruated. According to the Indonesian Demographic and Health Survey 2017, it was found that as many as 28.76% of women aged 30-49 years experienced menopause [1]. At that time, women experienced various changes associated with decreased ovarian function. Many changes have occurred, both long-term and short-term. One of the most common long-term changes is osteoporosis. World Health Organization (WHO) expressed that the blast of menopause in the following close a long time will be challenging to be forestalled. It is anticipated that in 2030 there will be 1.2 billion women over 50 years of age [2]. The greater part of the (roughly 80%) live in an emerging nation, also consistently menopause women populace increments by 3%. The prevalence of osteoporosis in the United States proves that 30% of women with menopause condition suffer from bone loss or osteoporosis. Menopausal women are at particular risk because estrogen loss will lead to bone mass loss much greater than one would expect based on age alone [3,4].

Current management of menopause, mostly uses hormone replacement therapy, especially estrogen, even

though it is mentioned that it can trigger an increase in the prevalence of malignancies in the breast and uterus. One of the components of cowpea bean, *Isoflavone*s, stated that it can be used as an alternative to estrogen. Phytoestrogens are compounds that have estrogenic activity whose molecular structure resembles 17-β-estradiol. An experiment by Filipovic, *et al.* showed that *Genistein*, a major soy phytoestrogen treatment improves the trabecular microarchitecture of the proximal tibia, induces histomorphometrical changes in thyroid glands, and decreases circulating thyroid hormone levels in orchidectomized rat model of male osteoporosis [5–7]. Several studies, such as that conducted in Korea and China stated that *Isoflavone*s supplementation has beneficial effects on bone markers and is effective in increasing limb bone density [8,9].

The Phytoestrogen Study Group (PERMI) Malang found that the local Malang non-soybean plants contained a lot of *Isoflavone*s. Related to all of these things, the authors are interested in examining the effect of non-soy bean or cowpea phytoestrogens on bone mass density which is examined through densitometry.

MATERIAL AND METHODS

Study Design

This was an experimental study with a double-blind randomized clinical trial applied to postmenopausal women in the city of Malang, where one sample was taken in each area in each sub-district of 10 sub-districts in the city of Malang. This research was conducted for 7 months, from April to November 2003.

Participants

Participants in this study were postmenopausal women in the city of Malang. The inclusion criteria in this study were postmenopausal women with a T-score ≤ 1, obtained from a densitometry examination of the left heel bone, and the results were converted into a T score. Exclusion criteria for subjects in this study were precocious menopausal women (<40 years old) either natural or artificial due to surgery, menopausal women who are currently or have used hormone replacement therapy, hormonal contraception, vitamin D, and drugs containing calcium. In addition, subjects taking anticonvulsant, cytostatic, heparin drugs, subjects with bone metabolic disease, mineral metabolism disease, or bone malignancy were not included in this study.

Randomization and Interventions

From all postmenopausal women who were examined, women with a T-score ≤ 1 were selected, so the results were 59 subjects. The subjects will be randomly divided into control and treatment groups, 30 subjects who are included in the control group and the rest, which is 29 people are include in the treatment group. Subjects in the control group will be given dry milk while the treatment group will be given 67.5 mg of *Isoflavone* for 24 weeks, consumed with a dose of 1 time a day. Participants do not know whether they are receiving a placebo or *Isoflavone*, and when the measurement arrives, we also do not know whether the participants are in the control or treatment group. There were no differences in the characteristics between both groups and they were in accordance with the predetermined inclusion criteria. Within 24 weeks of treatment, subjects will be subjected to densitometry examination, twice on the left heel bone with an interval of 3 months. The *Isoflavone* powder in this study was obtained using the HPLC (high-performance liquid chromatography) method. With this method, it will be known that the content of cowpeas is *Isoflavone*s.

Ethics

All techniques in this study were carried out in compliance with the appropriate manuals and regulations and were approved by the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

Statistical analysis

Statistical analysis was analyzed using SPSS Version 11.0 for Windows. We did ANCOVA (Analysis of Covariant) to analyze the differences between groups. P<0.05 was considered to indicate statistical significance.

Table 1. Subject Characteristics and Early Densitometry Measurements

Characteristics	Control group (n=29)		Treatment group (n=30)	
	Mean	SD	Mean	SD
Age (years)	61,31	6,65	59,73	6,73
Early T-score	-2,28	0,93	-2,53	0,93
Duration of amenorrhea	12,1	0,92	12,2	0,94
BMI	24,53	7,48	24,26	7,55
Coffee (cup/day)	0,86	1,04	0,75	0,98
Hijab (years)	6,23	9,35	5,14	9,39

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Table 2. The Average T Score of The Control and Treatment Groups During The Study

Group	Total	Initial T-score average (SD)	Average T- score 3 months (SD)	Average T- score 6 months (SD)
Control	29	-2,529 (0,93)	-2,742 (0,273)	-1,304 (0,262)
Treatment	30	-2,280 (0,93)	-3,487 (0,252)	-3,021 (0,252)
p value			0,065	0,000

Table 3. The Average Change in The Control and Treatment Groups

T-score	Control group (n=29)		Treatment group (n=30)		*p*
	Mean	SD	Mean	SD	
3 months mean T-score	-0,3690	0,76303	-1,2867	0,89239	0.006
6 months mean T-score	0,9517	1.12746	-0,7607	0,94921	0,000

RESULTS

A total of 59 subjects were included in this study. The initial characteristics of the subjects are shown in Table 1. The table shows the homogeneity of the subjects of this study. The bone density of all subjects was measured using densitometry and the results were converted into a T-score. The T-score of the study subjects was measured before the intervention, then measured again at the 3rd and 6th month of the study. The results of the T-score measurement during the study can be seen in Table 2. Based on the results of this study, it was found that the densitometry value were different between the treatment and control groups after 6 months with a p-value of 0.000 and were shown in Table 3. In addition, based on the results of the ANCOVA analysis, it was found that coffee was a confounding factor that had a significant effect in the 3rd month (p-value 0.043) (Table 4), but had no effect in the 6th month (p-value 0.944) (Table 5).

DISCUSSION

The research results listed in table 2 show the T-score value at the 3rd and 6th evaluations. From the magnitude of the p-value at 3rd month, which is 0.065, it can be interpreted that there is no clinical difference in the treatment and control groups. Meanwhile, in the 6th month, with a p-value of 0.000, a significant difference was seen due to the effect of treatment when compared to placebo. From table 3, it can be seen that the comparison of changes in T-score values between the treatment and control groups shows a very significant difference in both the 3rd and 6th month evaluations.

Comparison of changes in the T-score between the treatment and control groups showed a very significant difference in the 3rd and 6th month evaluations. This demonstrates the significance of administering cowpea powder to postmenopausal women, as evidenced by a decrease in the rate of bone loss and an increase in the rate of bone mass development in the 6th month compared to the control group. *Isoflavone*s, the main components of this study, are well known for their ability to modulate key signaling pathways that regulate bone resorption, such as decreased urinary deoxypyridinoline, collagen type 1 cross-linked N-telopeptide, and collagen type I cross-linked C-telopeptide, which also help in bone formation. [10]. In addition, this compound is able to increase the serum concentration of osteocalcin and alkaline phosphatase. In cultured osteoblasts, *daidzein* exhibits anti-resorptive activity via stimulation of osteoblast differentiation, increased estrogen expression of β receptors, and inhibition of osteoclastogenesis, the latter through the reduction of nuclear activator receptors factor-κB ligand (RANK-L)/osteoprotegerin ratio. Apart from osteoblasts, it is known in cultured bone marrow *Genistein* has the ability to reduce the activity of osteoclasts [10,11].

Osteoclasts are important cells regulators in bone resorption and they are derived from bone marrow monocytes/macrophages (BMMs). Increased bone loss observed due to estrogen deficiency in postmenopausal women suggests protective functions of estrogen on osteoclast activation and subsequent bone loss [12]. In osteoclasts, the mechanism of action is through non-genomic pathways, because osteoclasts have only a few estrogen receptors. The mechanism that can occur is its effects on the intensity of the secretion of osteoprotegerin (OPG) by osteoblastic cells. This can cause a decrease in the interactions of receptor activators for nuclear factor B ligand (RANKL) so that osteoclast activity can be minimized [13,14].

Genistein and *Daidzein*, the component of *Isoflavone* are able to increase alkaline phosphatase activity in cultured MC3T3-E1 pre-osteoblastic cells and also activate nuclear peroxisome proliferator-activated receptors (PPAR) and mediate the PPAR gene to modulate osteoclast function [15,16]. In addition, *Isoflavone* can cause a reduction in bone turnover and increased osteoblastic activity through inhibition of both tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-

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Table 4. The Results of Covariant Analysis (ANCOVA) of The Contribution of Confounding Variables to The Change in The T-Score of The 3rd Month

Variable	Median	Function	*p*
Age	0.00597	0.009	0.926
Amenorrhea duration	0.005224	0.008	0.931
BMI	1.296	1.899	0.175
Low calcium diet	1.800	2.638	0.112
Coffee	2.983	4.371	0.043
Exercise	0.767	1.124	0.295
Race	1.373	2.011	0.163
Sun exposure	0.02552	0.037	0.848
Hijab use	0.0003619	0.001	0.982
Treatment	5.803	8.502	0.006

Table 5. The Results of Covariant Analysis (ANCOVA) of The Contribution of Confounding Variables to The Change in The T-Score of The 6th Month

Variable	Median	Function	*p*
Age	1.597	1.442	0.237
Amenorrhea duration	4.004	3.614	0.065
BMI	2.496	2.253	0.141
Low calcium diet	0.927	0.837	0.366
Coffee	0,005498	0.005	0.944
Exercise	0.251	0.226	0.637
Race	0.184	0.166	0.686
Sun exposure	0.157	0.141	0.709
Hijab use	0,006029	0.005	0.942
Treatment	31.387	28.329	0.000

2), another function is to assist the activation of vitamin D [17,18].

This study is in line with research conducted by Chi et al, where the study involved 40 women for 6 months with 90 mg/day of *Isoflavone*s and caused an increase in a stronger descent of the concentration of ALP and a decrease of IL-6 and TNFα. This could improve some menopausal syndromes and was effective in increasing limb bone density[8]. Likewise, Harkness's randomized controlled trial of *Isoflavone* powder using a dose of 110 mg/day showed effective results in reducing bone resorption in postmenopausal women [19].

In this randomized, double-blind, placebo-controlled study, several risk factors were eliminated by research design techniques, such as: precocious menopause, drug users, and smokers. Other factors that cannot be eliminated by design due to the limited number of subjects will be eliminated by analysis (ANCOVA). This is then considered as a confounding variable in table 4 and 5 show that in the 3rd month (table 4), coffee consumption has a significant value of 0.043, but it is different in the 6th month (Table 5) where coffee does not appear to have a significant effect.

Coffee is still being debated as a risk factor for osteoporosis in postmenopausal women. According to research conducted in Korea and Taiwan, coffee consumption can lower the risk of osteoporosis while increasing T-scores in both men and women with postmenopausal conditions. This is due to several things, such as the estrogenic effect, antioxidant effect, and also the anti-inflammatory effect of coffee which might be due to the inhibition of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) expression in the inflammatory sites [20,21]. In contrast, a meta-analysis study conducted in Singapore comparing coffee and tea consumption found that drinking 4 cups of coffee per day is related to a higher risk of hip fracture, although a moderate intake may reduce risk in postmenopausal women. The in-vitro effects of caffeine on cells involved in bone metabolism suggest that caffeine intake may promote osteoporosis, and some but not all clinical studies support a modest adverse caffeine impact [22,23].

In this study, there are several limitations. The first limitation is that this study uses a bone density measuring device with a specificity level of 87.7% and a sensitivity of 90.2%, thus allowing for bias. In addition, the number of research subjects was small, and the side effects of cowpea powder were given in the form of vomiting and nausea. Despite these limitations, 59 participants in our study had high compliance (100% reached 80% compliance) with carefully limited *Isoflavone* over the study period.

According to the findings of this study, it can be stated that cowpea powder containing *Isoflavone*s is helpful in the treatment of osteoporosis. Compared to the control group given a placebo, there was a lower rate of bone loss (3rd month evaluation) and a higher increase

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in bone mass gain (6th month evaluation). Given the theoretical foundation outlined above, it is evident that administering cowpea-based *Isoflavone* phytoestrogens to postmenopausal women will minimize their risk of osteoporosis.

CONCLUSION

The provision of cowpea-based *Isoflavone* phytoestrogens has a positive effect on bone mass changes in postmenopausal women. In this study, there was a reduction in the rate of bone mass loss and an increase in bone mass development due to the administration of cowpea-based *Isoflavone* phytoestrogens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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