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Potential Of Soy Protein Isolate As Preventive Agent Of Osteoporosis In Ovariectomized Rats

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ABSTRACT :Objective : *The aim of this study was to determine potential soy protein isolate as a preventive agent of osteoporosis on improving bone strength in hipoestrogen condition by comparing the natural product of soy protein isolate with (ethinylestradiol.*
Material and Method *: This study used an experimental method with completely randomized design (CRD). A total of twenty-five 4-month old female Sprague Dawley rats were randomly assigned to five treatment groups (non ovariectomized; ovariectomized (OVX); ovariectomized+ estradiol 10 µg/kg/day (OVX+E2); ovariectomized + soy protein isolate 0.54 g/g/d (OVX+SPI1); ovariectomized+soy protein isolate 2.16 g/g/d (OVX+SPI2)). Rrats were euthanized after 30 days treatment and tibia bones were collected for the assesment of bone Ca and P levels and measure of bone strength using UTM (universal testing machine), and liver were collected to asses MDA levels. Bone strength is obtained by using a UTM tool with the principle of maximum strength in the weight-bearing bone by bone surface area.*
Result *: Soy protein isolate were increased bone calcium and phosphorus levels significantly (P <0.05) compared to all groups treatment.Ovariectomized rats + SPI diet alsoincreased bone strengthsignificantly up to 40.26% compared with the other groups (P<0,05). Ovariectomized rats + estradiol diet had higher level of Ca and P and greater of bone strength levels than in ovariectomized rats group. Dietary soy protein isolate with a 2.16 g/g/d dose were able to reducelevels of MDA (P<0,05).*
Conclusion *: Result of this studys suggest that soy protein isolate diet had potential as preventive agenton bone loss with demonstrated to elevate levels of boneCa and P and improved bone strength significantly. Soy protein isolate also hadpotential as an antioxidant activity by reduced levels of MDA in ovariectomized rats.*
KEYWORDS *–soy protein isolate, bone strength, ovariectomized rats.*

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I. INTRODUCTION

Estrogen has a variety of physiological effects on various cells and tissues. Estrogens increase of collagen in connective tissue, increase vitality and sexuality in women, increase levels of growth hormone (GH) and has a major role in the regulation of the bone remodelling process. (1) Estrogenis produced by ovaries and will be reduced in number after menopause. (2) Estrogen deficiency in both humans and animals raises a variety of issues, such as vasomotor dysfunction, skin atrophy, atrophy of the vaginal epithelium to osteoporosis. (3)

Osteoporosis is one of the signs of aging that is characterized by reduced bone mass density as a result of the release of excess bone mineral. (4) Osteoporosis may occur due to bone resorption speed is higher than the bone formation. (5) Estrogen plays an important role in bone remodeling process and will cooperate with osteoblast to build a new bone unit. (6)It is well documented by previous record that long term use of hormone replacement therapy in postmenopausal women may increase the risk of breast cancer.(7)

Phytoestrogens are non-steroidal compounds in plants that are structurally and functionally similar to 17-β-estradiol and have abilty for binding to estrogen receptors. (8) The ability of phytoestrogens in binding to estrogen receptor (estrogenic effect) make this compound widely used as therapy in hipoestrogen statue as addition to hormone replacement therapy. Isoflavone is one of the phytoestrogen compounds which have been widely known and it can be found in soybean. Soy protein isolates are products of soybean that have contain a high isoflavones which is about 97.43mg / 100g / bdd. Soy protein isolate has capable of lowering the speed of bone resorption without decrease bone density. (9) Long term- administration of soy protein isolate also able to increased bone density in mice and was found to affect postively bone metabolism. (10) Soy protein isolate

werereported reduce the loss of calcium on bone from mechanism calcium excretion through urine and decreased activity of bone resorption. (11)

Prevention of osteoporosis is necessary to reduce the risk of fractures and other bone diseases due to estrogen deficiency can be done in many ways including adminestering soy protein isolate in based diet. Present study has the objective to measure the potential of soy protein isolate as an agent of phytoestrogens in preventing the risk of osteoporosis. Primaryoutcame of this study is to see the ability os soy protein isolate to improve bone strength and bone mineral content and secondary outcame is to see the ability of soy protein as an antioxidant compounds in order to decrease levels of oxidative tissue may has caused by estrogen deficiency.

II. MATERIAL AND METHODS

2.1 Animal Care

Twenty-five female rats Sprague dawley, aged 13 weeks were used in this study. They were kept at room temperature at least 10 days prior to surgery. Rats were acclimated to a standard laboratory diet and tap water freely for 10 days. After acclimation rats were divided by initial body weightinto five blocks of five rats each, using a randomized complete design.

2.2 Experimental desgin and dietary assesment

Twenty of twenty-five female rats were ovariectomized by following standard operating procedure based in Animal welfare proceed. Rats were divided into five groups of treatment after 30 days from ovariectomy : (group 1 : non ovariectomized rats; Group 2: ovariectomized (OVX) rats ; Group 3 : ovariectomized rats+ estradiol 10 µg/kg/day (OVX+E2); Group 4 : ovariectomized rats + soy protein isolate 0.54 g/g/d (OVX+SPI1); Group 5 : ovariectomized rats+soy protein isolate 2.16 g/g/d (OVX+SPI2)). Estradiol and soy protein were administered orally to ovariectomized rats 30 days after surgery. All rats in all groups of treatment had access to water freely. Rats were euthanized 30 days after treatment and tibia bones were collected for the assesment of bone Ca and P levels and measurement of bone strength using UTM (universal testing machine), and liver were collected to asses of MDA levels.

2.3 Bone Calcium and Phosporus

Analysis of bone calcium levels were calculated using Wet ashingmethod (Reitz et al 1960). Tibia bone weighed approximately up to 1 gram and then added of some nitric acid for 1 hour at acid storage cabinet. Bone had heated above the bath for 4-6 hours at acid cabinet, then left overnight. Added 0.4 mL of sulfuric acid and then heated again up to an hour on the bath until the solution is reduced and becomes more concentrated. The addition of various acids in a sample which was heated to form a color change from brown to amber approximately one hour. After a change of color, bone was heated again for 10-15 minutes. The samples were cooled and distilled water is added along with 0.6 mL of hydrochloric acid. Dissolving the samples returned by way of re-heated for 15 minutes. The precipitate is filtered with glass wool, then calcium was measured using atomic absorption spectrophotometer (AAS).

2.4 Bone Strength Testing

Left tibia bone is separated from the soft tissue, then cut to a size of 15 mm and trimmed both ends. An inner diameter and outer diameter on both ends of the tibia was measured using a digital caliper before testing. Then bone was placed on the tool Universal Testing Machine (UTM) Instron. Testing bone strength in this study using the compressive strength test with the analysis of the type of loading press, namely press perpendicular, with bone strength testing formula:

$$Fc = \frac{P}{A}$$

Ket :
Fc :Bone Stength of Holding Load (kg/cm^2)
P : Load (kg)
A :Surface area (cm^2)

2.5Liver MDA levels

Rat liver was washed with 0.1% buffersalineand then divided in two parts. One section was weighed, wrapped in aluminum foil and stored in a freezer at -20 ^{0}C. then liver was crushed using a pestle (crushed in a cold state), then added 1.25 ml of phosphate buffer containing 11.5 g / L of potassium chloride in the cold state of pH 7.4 and then stored at 5 ^{0}C. Then aliquot was centrifuged at 4000 rpm speed for 10 minutes, turbid supernatant obtained and centrifuged again at 4000 rpm speed for 10 minutes, 1 ml of the clear supernatant was taken and added to 1 ml of a mixture of hydrochloric acid cold solution of 0.25 N (2 , 23 ml of concentrated hydrochloric acid / 100 ml) containing 15% trichloroacetic acid (w / v); 0.38% and 0.5% tiobarbiurat acid

butilathidroksitoluen). A mixture of the supernatant and solution were heated in an incubator at a temperature of 80 ^{0}C for one hour, then cooled with water and then centrifuged at 3500 rpm speed for 10 minutes. Furthermore, the values of liver MDA levels measured using the absorbance at a wavelength of 532 nm.

2.6 Stastical Analysis

Data were expressed as mean ± SEM. One way analysis of variance was performed to analyze whetheher there were significant differences among the groups and followed by Duncan test using SPSS program version 16.0. The values were considered statistically significant at P<0,05.

III. RESULT

3.1 Bone calcium and phosporus

The performed experiment demonstrated that dietary soy protein isolate on both doses (0.54 g/g/d and 2.16 g/g/d) were significantly (P<0,05) increased bone calcium and phosporus levels compared to the other treatments (Table 1). Ovariectomized rats + estradiol diet group hadhigher bone calcium and phosporus compared with ovariectomized rats group, but lower compared with normal rats (non ovariectomized group). Increased levels of bone calcium above 8.57% and bone phosporus above 2.79% in ovariectomized rats + estradiol diet group compared with non-treatedovariectomized rats (OVX).

Table 1. Effect of Soy Protein Isolates on bone Calcium and Phosphorus levels.

Groups of treatment	Parameter	
	Bone Calcium (%)	Bone Phosporus (%)
Normal	23.107±1.277026^b	28.182±8.4597 ^{b}
OVX	20.868±2.459268^b	27.326±3.2861 ^{b}
OVX + E	22.657±1.203578^b	28.091±4.6339 ^{b}
OVX + ISP1	56.124±4.099943^a	44.227±22.15 ^{a}
OVX + ISP2	58.295±6.641064^a	32.742±12.373 ^{a}

Normal = non ovariectomized rats, OVX = non-treated ovariectomized rats, OVX + E = ovariectomized rats + 10µg /kg/day, OVX + SPI1 = ovariectomized rats + 0.54 g/g/D, OVX + SPI2 =ovariectomized rats + 2.16g/g/d. Values are least square means ± SEM, values with different superscripts are significantly different (P<0,05).

2.2 Bone Strength

As expected, dietary soy protein isolate of2.16g/g/d(P<0,05) increased levels of bone strength significantlycomparedwithadministration ofestradiolandsoy protein isolatewithlower doses(Fig.1). An increaseof40.26% of bonestrengthin ovariectomized ratsby administeringthe soy protein isolateof2.16g/g/dgreater than normal rats (non ovariectomizedrats group). Estradiol diet was increased levels of bone strength compared with non-treated ovariectomized rats group. Non-treated ovariectomized rats (not given estradiol or SPI) had a lowest levels of bone strength among all group.

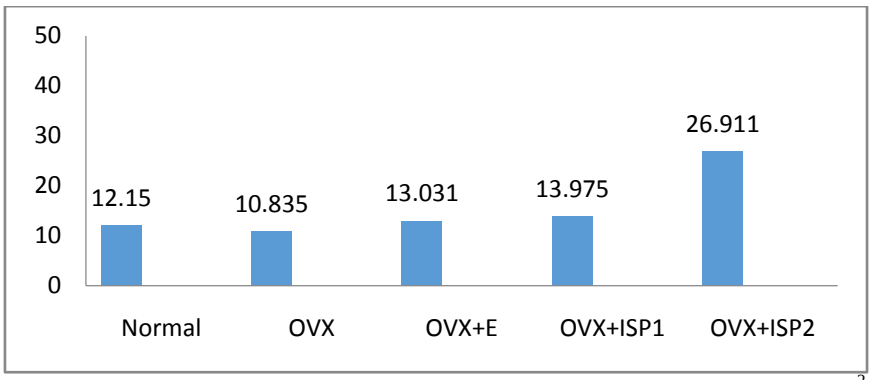

Figure 1 Effect of dietary soy protein isolate in improving bone strength (kg/cm^2)

Normal = non ovariectomized rats, OVX = non-treated ovariectomized rats, OVX + E = ovariectomized rats + 10µg /kg/day, OVX + SPI1 = ovariectomized rats + 0.54 g/g/D, OVX + SPI2 =ovariectomized rats + 2.16g/g/d. Values are least square means ± SEM, values with different superscripts are significantly different (P<0,05).

2.3 Liver MDA levels

Liver MDA levels in all groups are presented in Table 2. Liver MDA levels in ovariectomized rats + estradiol diet had higher values among all groups (7.329±2.44) and significantly(P<0,05) different from ovariectomized rats + soy protein isolate diet. Data showed that was decreased levels of liver MDA from estradiol diet to soy protein isolate diet.

Table 2. Effect of dietary soy protein isolate in Liver MDA levels

Groups of treatment	Parameter Liver MDA levels (µg/gsampel)
Normal	6.532 ± 1.8776^{ab}
OVX	4.729 ± 2.4587^{b}
OVX+ Estradiol	7.329 ± 2.4425^{a}
OVX+ ISP1	5.785 ± 0.666^{ab}
OVX+ ISP2	4.079 ± 0.3937^{b}

Normal = non ovariectomized rats, OVX = non-treated ovariectomized rats, OVX + E = ovariectomized rats + 10µg /kg/day, OVX + SPI1 = ovariectomized rats + 0.54 g/g/D, OVX + SPI2 =ovariectomized rats + 2.16g/g/d. Values are least square means ± SEM, values with different superscripts are significantly different (P<0,05).

IV. DISCUSSION

Dietary soy protein isolate administered to ovariectomized rats increased bone calcium and phosporus than the other groups. Bone calcium levels is assossiated with sulfur amino acids and retention of calcium itself. Proteins contained in soybean is a type of protein that have low sulfur containing amino acids and a high potassium.It has been reported that dietary protein increase urinary calsium excretion and can lead into calsium imbalance. (12) Lower sulfur amino acid which were contained in soybean could reduce loss of calsium through urine so it could increased total calsium in bone. It has also been reported by other researchers that administered of soy protein isolate decreased levels of calcium excretion through urine in postmenopausal women. (13) Women menopause supplemented with soy protein isolate proved to show a significant decrease in Ca excretion in the amount of above40mg/d when compared with the control. In fact, previous records claimed that the activity of calcium re-absorption in renal could be expected to protect bone mass loss of up to 1% / year. (14)

Dietary soy protein isolate administered to ovariectomized female rats on both dose (0,54 g/g/d and 2:16 g/g/d) also enhanced bone phosphorus levels significantly. As with calcium, phosphorus is also an essential mineral that plays a role in many bodily functions and systems, above 85% of phosphorus contained in the body of bones and teeth. Increased levels of bone phosphorus on SPI diet is believedto assosiate with ability of SPI as estrogen-like (isoflavone). Soy protein isolate contain high of isoflavone (97.43 mg / 100g bdd) when compared with the other processed soy products. (15) The isoflavones in soy have the ability to bind to estrogen receptors in the body. Estrogen is an inhibitor in the process of reabsorption of Ca and P in bone, acts on the kidneys to increase the reabsorption of Ca and P in kidney tubules and mineral reabsorption back into the duodenum. (16) The increase in mineral reabsorption in the renal tubules and duodenum is automatically lowered Ca and P loss through the urine so that the storage of calsium and phosporu in bone aregetting higher.

Bone strength values in the present study is obtained by calculating the maximum ability to withstand loads up the bones in until they were broken. Value of bone strength is influenced by bone microarchitecture and degree of mineralization of bone tissue. (17) Dietary of soy protein isolate with highest doses can increase bone strength of up to 40.26% when compared to normal rats that were not given any treatment or diet. The main compounds in soy isoflavones are genistein and daedzin which have a great influence on the increase in bone strength. According to previous records, both genistein and daedzin have capability of stimulating proliferation, differentiation and activation of osteoblasts through its binding to estrogen receptors on bone. (18) Increased activity of osteoblasts which initiate the bone formation and bone mineralization thus directly could increase the bone strength. Isoflavones in soy protein isolates also had an effect on osteoclasts. Other previous records reported that low doses of genisteinthat given by invitrocould reduced the number of osteoclasts cells in bone marrow cultures. (19) Osteoclasts are cells that play a role in inducing bone resorption process. Excessive bone resorption process will lead to loss of bone minerals and bone microarchitecture changes and the bone density will be reduced. Reduced bone density will decrease the strength of the bones in the body.Administered of soy protein isolate containing isoflavones have been shown to maintain bone mineral loss in excess bone mineral density in order that increasing bone strength.

Dietary soy protein isolate in highest dose showed to decrease MDA levels significantly compared with esradiol diet. This suggests that a decline in the activity of free radicals thereby reducing oxidative damage to the tissue. High content of isoflavonesin soy protein isolate that acts as an antioxidant agent and have capable of neutralizing free radicals. Potential of isoflavones as an antioxidant also confirmed by other researchers who reported that the administration of isoflavone compounds have ability to reduce levels of MDA in rats with stress state of the environment. (20) The decrease of MDA in stress organism is caused by the reaction of isoflavones that prevent lipid peroxidation reaction. Isoflavones will donate one of its electron to the free radical compounds that have a shortage of electrons in order that free radicals will be more stable and will not attract electrons from other tissues, thereby reducing the occurrence of damage to the tissue. (21) This makes the isoflavones in soy protein isolates can prevent tissue damage due to the effects of free radicals.

V. CONCLUSION

We concluded that soy protein isolate can be used as agents in the prevention of the occurrence of osteoporosis in estrogen deficiency states (menopausal women) by increased mineral in bone and improved bone strength. Further research is needed to determine the effect of soy protein isolate on various networks with more diverse marker.

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