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CTx serum level in mice post-ovariectomy after administration of sweet potato extract (Ipomoea batatas) is lower than without administration of sweet potato extract (Ipomoea batatas)

Tungka Melissa PJ,* I Ketut Suyasa, Anak Agung Wiradewi Lestari, Putu Astawa

ABSTRACT

Background: Osteoporosis is a systemic bone disease characterized by a decrease in bone mass. Postmenopausal osteoporosis is the most common osteoporosis. In postmenopausal osteoporosis, the increase in bone resorption is due to estrogen deficiency. Phytoestrogens are natural substances that can affect estrogenic activity. One of the plants that have phytoestrogens substances is sweet potato (Ipomoea batatas). Sweet potato (Ipomoea batatas) contains anthocyanin that acts as phytoestrogens agent to prevent osteoporosis in postmenopausal women.

Aim: This study aimed to determine the effect of sweet potato extract administration toward CTx level.

Methods: An experimental study of post-test control group design was done using 36 female Wistar mice. All mice underwent ovariectomy and were divided into two groups. The first group was given sweet potato extract 400 mg/day, while the second group did not receive any sweet potato extract. In the fourth week, the treatment effect was assessed by examining serum C terminal telopeptide (CTx) level that was taken from mice blood.

Results: Statistical analysis found that the mean serum C terminal telopeptide (CTx) levels in mice receiving sweet potato extract were lower than those without the sweet potato extract. Independent t-test that was done on the sample found significant differences with $p = 0.000$ ($p < 0.05$).

Conclusion: Administration of sweet potato extract on post-ovariectomy mice resulted in a lower level of serum C terminal telopeptide (CTx) than those without sweet potato extract.

Keywords: Osteoporosis, Sweet Potato Extract, ovariectomy, C Terminal Telopeptide.

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Department of Orthopaedic and Traumatology, Faculty of Medicine Udayana University – Sanglah Hospital, Bali, Indonesia

*Correspondence to:
Tungka Melissa PJ Department of Orthopaedic and Traumatology, Faculty of Medicine Udayana University – Sanglah Hospital, Bali, Indonesia
melissapingkan@yahoo.com

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INTRODUCTION

Ovariectomy in an animal can lower the estrogen levels by switching off the function of ovaries as the primary producer of estrogen. The removal of ovaries can lead to decreased concentrations of estrogen in blood circulation.

Ovariectomy in mice serves as an animal model of estrogen deficiency which resembles the condition in menopause women. A decrease in estrogen level resulted in decreased intestinal calcium absorption and increased calcium excretion through the kidneys. In bone, estrogen works to increase bone resorption, decreasing osteoblasts formation, and bone density. Higher bone resorption compared with bone formation results in osteoporosis or brittle bones.

The functions of estrogen are to increase intestinal calcium absorption and reabsorption of renal tubular calcium. Estrogen deficiency in an individual with ovariectomy causes hypocalcemia related to the decrease in calcium absorption in the intestine.

Ovariectomy treatment in mice or menopausal women can lead to a decrease in estrogen hormone, calcium reabsorption in the kidneys as well as an increase in urinary calcium excretion.

The decrease in estrogen hormone after ovariectomy is often associated with an increase in bone resorption, decrease of bone density and increase the risk of fractures. A decrease in estrogen resulted in decreased intestinal calcium absorption and increased calcium excretion through the kidneys, even low calcium level can increase bone calcium resorption and loss of bone mass in mice after ovariectomy. In the bone, estrogen works by decreasing osteoclasts formation and osteoclasts activity to resorb bone.[1,2] Estrogen may increase osteoblasts formation, and osteoblasts function in bone-forming,[3-5] as well as to control osteoblasts and adipocytes in the bone marrow.[6,7] Estrogen also works on the bone indirectly through the inhibition of parathyroid hormone activity,[8,9] and activation of the kidneys to convert vitamin

D2 (25- dihydroxyvitamin D3) to vitamin D3 (1,25-dihydroxyvitamin D3).9 Aside from maintaining blood calcium levels within the normal range, vitamin D3 supplement also works in the process of skeletal mineralization.[10] Administration of vitamin D3 supplement can increase plasma vitamin D3,[11,12] stimulates transcellular transport of calcium, stimulates intestinal calcium absorption, and reabsorption in renal tubular calcium,[13-17] stimulate bone formation and increase bone density by stimulating osteocalcin synthesis of osteoblasts and bone matrix mineralization process.[10,18-20] It is also important to note that the consumption of vitamin D3 supplement in high doses may result in hypercalcemia, hypercalciuria, and increase calcium resorption in the bones.[21,22]

One of the most common bone diseases today is osteoporosis or known as a bone loss. There are some varieties of pharmacological and herbal products produced and researched to fulfill people's desire in preventing and treating bone loss/osteoporosis. One that began to develop in society is the use of herbal products for the prevention and treatment of bone loss/osteoporosis. The use of herbal extracts or medicines is preferred because the risk of its side effects is lower than synthetic medicines. However, the use of herbal medicines still requires further investigation because many herbal medicines in circulation do not have a clear evidence-based to support their claims.

Estrogen replacement therapy or ERT has long been used as a way to prevent and treat postmenopausal osteoporosis. Estrogen primarily works by reducing bone resorption. Hormonal injection to replace estrogen (Estrogen Replacement Therapy – ERT) is commonly prescribed to menopausal women to cope with symptoms that occur in post-menopause and to prevent chronic disease such as osteoporosis. Synthetic estrogen mixture was given as a therapy to replace expensive hormone therapy and have dangerous side effects. Synthetic estrogen can disrupt blood clotting processes, interfere with enzymes that work in the liver, causing uterine and endometrium bleed, nausea and vomiting.[23] Administration of estrogen orally, transdermal, or as an implant has been shown to increase bone density significantly.

CTx or Cross link C-telopeptide is a bone-specific protein and a cluster of unique amino acids that act as a biochemical indicator of bone resorption processes that exhibit osteoclast activity in bone. CTx is one of the latest developments of specific and sensitive bone biochemical markers to identify the rate of formation and bone resorption in some bone metabolic disorders, especially osteoporosis. CTx as bone biochemical markers can provide an overview of the ongoing remodeling process. This examination includes bone resorption markers by osteoclasts and bone formation markers by osteoblasts. Evaluating bone biochemical markers serves several purposes such as monitoring and assessing treatment response, diagnosis of patients with osteoporosis risk, finding etiology of reduced bone mass, choosing treatment, monitoring patients with corticosteroid treatment, studying the pathogenesis of osteoporosis, estimating the bone loss in postmenopausal women, and estimating the incidence of osteoporotic fractures.

It is wise to use phytoestrogens therapy as an alternative for menopausal women because it is proven to be safe. Phytoestrogens are natural substances that can affect the body estrogenic activity. One of the plants that have phytoestrogens substances is sweet potato (Ipomoea batatas). Consumption of sweet potato (Ipomoea batatas) can increase bone strength in ovariectomized mice. Sweet potato (Ipomoea batatas) contains anthocyanin that acts as a phytoestrogens agent to prevent osteoporosis in menopausal women.[23]

Anthocyanins are the pigments found in sweet potato (Ipomoea batatas). The anthocyanin content contained in the sweet potato is much higher than that found in other foodstuffs. Even as a food dye, the anthocyanin of sweet potato is very stable against warming and UV radiation.[24]

Currently, there is no research to show the anthocyanin effects of sweet potato (Ipomoea batatas) on bones. The estrogenic effect in sweet potato (Ipomoea batatas) becomes an interesting phenomenon and an opportunity to develop a cheap and safe alternative therapy to increase bone density and can be used to prevent and treat postmenopausal osteoporosis.

There are several methods for assessing the effects of postmenopausal osteoporosis therapy in mice, one of them is the examination of bone resorption markers. One of the bone resorption markers is Cross-linked C-terminal telopeptide (CTx).[25]

MATERIALS AND METHOD

This research is experimental research using post-test only control group design with mice as the subject. In general, the factors affecting bone resorption in mice are similar to human. Those factors are also divided into intrinsic and extrinsic factors. Intrinsic factors that affect the rate of mice bone absorption include strains, age, gender, weight, hormonal and specific diseases. Meanwhile, the extrinsic factors include nutrition, physical activity, certain drugs or medicines, and environment. Ovariectomy was performed to create the condition of postmenopausal osteoporosis in mice.

Table 1 The mean CTx serum level in each group

Variable	Groups		
	Post-ovariectomy (n=36) (Mean ±SD)	Treatment with sweet potato extract (n=18) (Mean ±SD)	Control without sweet potato extract (n=18) (Mean ±SD)
CTx serum level	1.72 ± 0.64	1.69±0.934	4.11 ± 0.424

Table 2 The normality test of research variable data using Shapiro-Wilk test

Variable	Groups	N	P	Status
CTx serum level	Treatment	18	0.978	Normal
	Control	18	0.934	Normal

Table 3 Comparison of post-test mean CTx serum level between treatment and control group

Variable	Groups		95% of CI	P-value
	Treatment with sweet potato extract (n = 18)	Control without sweet potato extract (n = 18)		
CTx Serum level	1.69±0.934	4.11±0.424	11.23-4.32	0.000

There are several methods for assessing the effects of postmenopausal osteoporosis therapy in mice, one of them is the examination of bone resorption markers. One of the bone resorption markers is Cross-linked C-terminal telopeptide (CTx). A random sample of 36 mice was chosen. The selected sample was divided randomly into two groups, both groups underwent ovariectomy, 1 group was given sweet potato extract, and the other group was only given water. The CTx (data) measurement was done a day after the treatment ended as well as blood was taken from the orbital sinus from one eye of the mice. Approximately 3 cc of blood was taken using hematocrit pipette and was stored in a sample tube with no EDTA. Samples were examined for CTx level after treatment in the laboratory.

RESULTS

The mean CTx serum level in post-ovariectomy mice is 1.72 ± 0.64. The mean CTx serum level in the treatment group with sweet potato extract is 1.69 ± 0.934, meanwhile in the control group is 4.[11] ± 0.424.

This analysis aims to generalize the results of the research to the population. The inferential statistical test used in this study is independent t-test if the data is normally distributed and the data variant is homogeneous. Assessment of test result using 95% of CI and p-value at a significance level of 0.05.

Normality Test

The research variables in the treatment and control groups were tested for normality test. Due to the sample size of 36 (n < 50), the normality test used was Shapiro-Wilk test. Homogeneity test of data variance was done using Levene's test.

The table above shows that the data of CTX serum level is normally distributed, with the p-value > 0.05

Independent T-Test

For numerical variables with normal data distribution, an independent t-test was performed to test the significance of two unpaired groups. Comparison of the post-test mean serum level of each group was made to determine the effect of each variable in the treatment and control groups.

The table shows that CTx serum level in the treatment group was lower than in the control group, and the mean difference between treatment and control group was statistically significant with the value of p=0.000 (p<0.05).

DISCUSSION

The research data has been processed and analyzed using statistical method and in accordance with the research hypothesis that has been made. The data were interpreted to determine the factors that influence the results of this study.

This research was aimed to determine the effect of administration of sweet potato extract toward CTx level. It was performed on female, white mice (Wistar type), six months old with 200 grams in weight, and in a healthy condition without defect.

There are 36 mice in this research which was divided into two groups, control and treatment group, each consisting of 18 mice. This study is the first research done to determine the effect of sweet potato extract toward CTx serum level in mice post-ovariectomy.

The statistical test shows that the mean of CTx serum level in the treatment group was lower than in the control group and the normality test shows that the CTx serum level in mice with sweet potato extract and that without sweet potato extract was normally distributed, in which the value of p is > 0, 05. An inferential test using independent t-test showed a significant difference between the two groups (p<0.05). These results show that the administration of sweet potato extract can decrease CTx serum levels in post-ovariectomy mice.

The mean of CTx level in post-ovariectomy mice was 1.72 ± 0.64, in the treatment group was 1.69 ± 0.934, and in the control group was 4.11 ± 0.42. The study of Sreeja and Anju (2010) proves that sweet potato (Ipomoea batatas) has an estrogenic effect in vitro.[26] The sweet potato (Ipomoea batatas) contains a phytoestrogen named isoflavones. The estrogenic activity of isoflavone is related to the isoflavone structure which can be transformed into equol. Equol has a phenolic structure that can bind to estrogen receptors. Isoflavones can bind both to estrogen-α receptors and estrogen-β receptors, but the bond with estrogen-β receptors is stronger.[27]

The study conducted by Tasmiatun et al. proved that administration of sweet potato extract with doses of 116, 232, and 463 mg/kgBW for 30 days did not increase bone calcium in ovariectomized mice. Perhaps, further studies of sweet potato extract should be performed again in mice with higher doses and longer time.[23]

Sweet potato is one of the plants that contain phytoestrogens. Phytoestrogens are a natural compound found in some plants that have a similar chemical structure as estradiol, an endogenous estrogen. Phytoestrogens can bind to estrogen receptors. Thus they have an estrogenic effect. The benefits of phytoestrogens are its role as SERMs. In specific tissues (bone and cardiovascular) it works as estrogens, while in other tissues (mammae, ovary, and endometrium) it works as anti-estrogens.[28,29]

Research on the effects of phytoestrogens on osteoporosis began with an observation that the prevalence of osteoporotic fractures in Asian women is lower than in European women. This phenomenon is related to the Asian women diet which has more fruits and vegetables rich in phytoestrogen than European women. Animal research also proves that phytoestrogens can increase bone formation and decrease bone resorption. Based on the meta-analysis conducted by Ma et al. toward nine studies in mice, it was found that flavonoids significantly decreased the excretion of deoxypyridinoline (DPD) in urine and increased bone alkaline phosphatase.

Observational and experimental studies in humans have also proved that phytoestrogens can increase bone mass. For example, an experimental study in England that compared the bone density of women who drank tea contain flavonoid and placebo, it was found that women who drank tea contain flavonoid had a higher bone density.[30-32]

The above studies supported the results of this research which shows that CTx serum level in the treatment group was lower than in the control group, and the mean difference between treatment and control groups were statistically significant with the value of p = 0,000 (p <0.05).

CONCLUSION

The CTx serum level in post-ovariectomy mice given sweet potato (Ipomoea batatas) extract was lower than those without sweet potato (Ipomoea batatas) extract.

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