Estrogenic Effect Ethanol Extract Corn Silk (*Stigma maydis*) on Bone Density and Histology Femur Profiles in Ovariectomized Rats Female Sprague Dawley Strain

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Abstract

Osteoporosis in menopause woman is caused by estrogen deficiency which plays an important role in bone formation. Corn silk (Stigma Maydis) contains stigmasterol, a phytosterol compound predicted to act as phytoestrogen. The aim of this research is to observe the activity of Corn Silk as the source of phytoestrogen by in vivo study in ovariectomized rats. Bone density analysis was examined by using x-ray. Meanwhile, histological profile of bone matrix was determined by HE-staining microscopic observation. Affinity of stigmasterol to ER (α,β) were evaluated by molecular docking. The results showed that treatment of EECS after ovariectomy has not been able to increase bone density compared to the control group OVX. Moreover, histological observation of bone matrix showed that EECS performed improvement effect compared to was observed in the administration of estradiol. Docking between stigmasterol and ER (α,β) gave the docking score which are almost the same as that seen in docking with estradiol. In summary, EECS produced positive effects on bone density in estrogens-deficient OVX rats by reducing bone resorption. Therefore, EECS may also prove to be helpful in preventing osteoporosis in postmenopausal women whose estrogen is insufficient.

Keywords: osteoporosis, phytoestrogen, corn silk, stigmasterol

INTRODUCTION

The estrogen hormone is one of the important hormones of women. At menopause, estrogen levels decrease to around 50% from the premenopausal concentration (Burger, *et al*., 2007). The estrogen hormone deficiency contributes to a decrease in bone density, redistribution of subcutaneous fat to visceral area, an increased risk of cardiovascular disease and reduced quality of life. Decreased levels of estrogen (17β-estradiol) accelerates differentiation of osteoclasts so that it becomes one of the main causes of osteoporosis in postmenopausal women (Carr, 2003; Pacifici 2006). Osteoporosis, the most common bone disease characterized by decreased bone mineral density (Adlercreutz, *et al*., 1992) as well as an increased risk of bone fractures. Estrogen deficiency induces an imbalance in *bone turnover*, *i.e.*, bone resorption by osteoclasts exceeds bone formation by osteoblasts (Rodan and Martin, 2000; Teitelbaum, 2007). The solutions offered today is through Hormone Replacement Therapy (HRT), but prolonged use can cause dangerous risks, such as breast breast cancer (Chlebowski, *et al.*, 2009).

Corn hair (*Stigma maydis*) is an organic waste and available in abundance (Maksimovic, *et al*., 2005) and is known to be a source of phytoestrogens through stigmasterol compounds. Therefore, this study aims to explore the potential of corn hair ethanolic extracts (HCEE) as a source of phytoestrogens to treat osteoporosis. Through molecular testing can be seen docking interaction between compounds stigmasterol in corn hair with estrogen receptor α and β. Studies in vivo through observing macroscopically and microscopically HCEE used to observe the effect on rat femur bone density profile terovariektomi.

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Painting hematoxylin-eosin (HE) is performed to see the profile of histology of bone matrix through observation *canalis haversi* that became one of the initial parameters of bone resorption process. Thus, research is needed to explore the potential of corn hair ethanolic extracts as phytoestrogen agents which will be used as reference in the development of corn hair as agents of prevention of osteoporosis in postmenopausal women.

RESEARCH PURPOSES

This research aims to determine the potency of HCEE in preventing the onset of osteoporosis in female *Sprague Dawley* ovariectomy rats. In this research, carried out observations of the femur bone density and bone matrix and histological profiles supported by molecular docking test to determine the interaction of compounds stigmasterol in corn hair with estrogen receptors (ERα, ERβ).

RESEARCH METHODS

Materials Testing

Corn hair test material collected from the area Purwomartani, Sleman, Yogyakarta. Chemicals such as ethanol 70%, CMC Na, distilled water, formaldehyde, 0.9% NaCl, β-estradiol, corn oil, cholesterol kit standard (DSI, Germany), 3/0 Meiyi® Plain catgut sutures, antibiotics Enbatic®.

Extraction of Corn Hair

Determined corn hair powdered and dried in Unit 2 Department of Pharmaceutical Biology UGM. Dry powder corn hair is extracted by reflux method using ethanol 70%. Reflux is performed at 70°C for 90 minutes. Subsequently, the extract was filtered and condensed by rotary evaporator to obtain a thick extract.

Qualitative Analysis

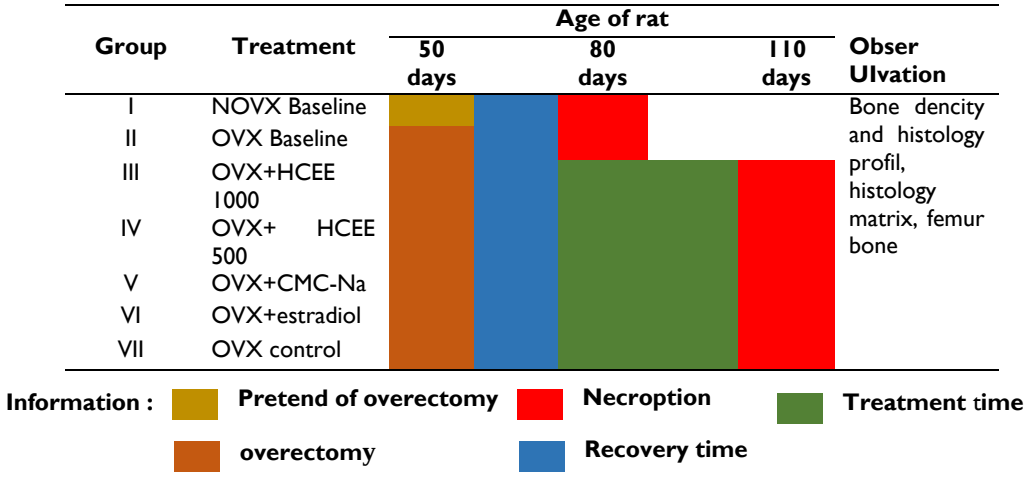
Identification of compounds stigmasterol in corn hair ethanolic extract (HCEE) using thin layer chromatography (TLC) with the stationary phase silica gel 60 GF254 plate and the mobile phase toluene: acetone: glacial acetic acid (3: 3: 0.15). TLC visualization is done with spray Liebermann-Burchard reagent LP and heated. Then observed under visible light, UV 254 nm, and 366 nm UV.

Molecular Docking

Data structure of ER-α (PDB ID: 2OCF) and ER-β (PDB ID: 4J24), which became the target protein docking downloaded from http://www.pdb.org then processed using software YASARA to regulate the environmental conditions in accordance with the physiological human. Stigmasterol structure can be obtained through the existing literature is then drawn using software MarvinSketch, so the protein and ligand is ready to be tested using a molecular docking PLANTS. Then put the data on the target protein and ligand in the form PLANTS configuration file and compared scores obtained between the target protein complexes with ligands the original and the target protein complexes with stigmasterol. Then do the docking visualization using software MOE.

In Vivo Test

This study used a total of 35 female Sprague-Dawley rats aged about 50 days. Mice created in estrogen deficiency states with a model of ovariectomy (making the ovaries of mice). These mice were then grouped into seven test group and the division of the group is in accordance with the following design.



Data Analysis

The affinity of ligand-receptor interaction was determined by molecular docking with parameter of docking score. The lower docking score indicated strong and stable interaction. Strong interaction of stigmasterol with estrogen receptor has potency to generate estrogenic effect. Macroscopic analysis of femur matrix density was identified using X-ray photo. While, microscopic analysis was done through observation of bone preparation.

RESULTS AND DISCUSSION

Preparation and Qualitative Screening

The coarse powder (739.75 g) of the corn silk was extracted with ethanol. The extract was concentrated using rotary evaporator to yield a viscous extract (22.5%). Detection of stigmasterol in corn hair ehanolic extract (HCEE) was conduted by thin layer chromatography (Fig. 1).

The plate showed between stigmasterol and CHEE had similar hRf value of 60 indicated that compound in CHEE had similar polarity with stigmasterol. Then, chromatogram was sprayed with Liebermann-Burchard LP reagent and observed under UV. Spot between stigmasterol and plat had same fluorescent. Thus, it can be summarized CHEE probably had stigmasterol as its compound.

Molecular Docking

The interaction affinity of stigmasterol and estradiol toward estrogen receptor can be predicted through molecular docking with the parameter of docking score (Table1). Molecular docking also supported by visualization of amino acid recidues of binding site (Fig. 2).

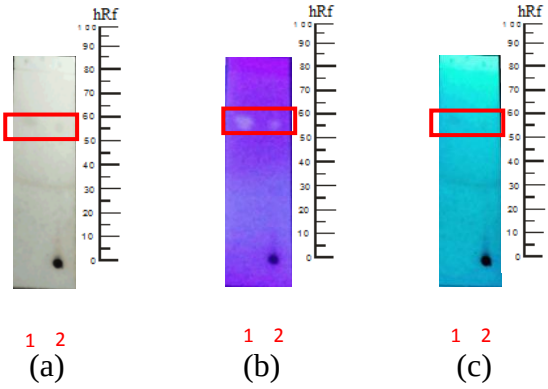
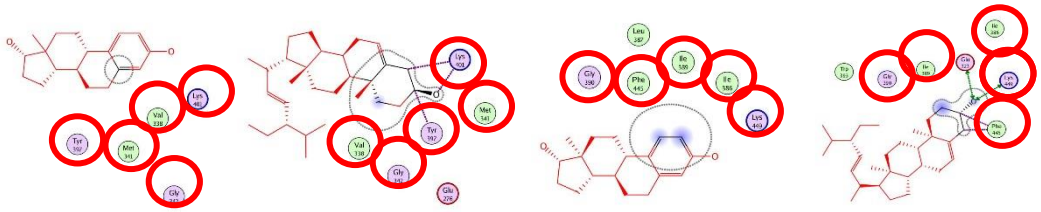


Figure 1. TLC Profile of CHEE. Extract was eluted using silica gel as stationary phase and toluen : aceton : glacial acetic acid (3 : 3 : 0.15) as mobile phase. Plat was observed under (a) visible light; (b) UV 366; (c) UV 254. Red boxes showed possibility of stigmasterol in CHEE with hRf value of 60. 1 : Stigmasterol and 2. CHEE.

Table 1. Docking score between stigmasterol and estradiol toward estrogen receptor.

Ligan	*Score docking*	
	ER-α (2OCF)	**ER-β (4J24)**
Estradiol	-94.3455	-96.1768
Stigmasterol	-88.713	-94.0201
RMSD	0.2738	0.4054



(a) (b) (c) (d)

Figure 2. Visualization of Binding Site. (a) ER- α (2OCF) and Estradiol; (b) ER- α (2OCF) and Stigmasterol; (c) ER- β (4J24) and Estradiol; (d) ER-β (4J24) and Stigmasterol. The circles showed the similar amino acid of binding site.

Docking score of stigmaterol with ER-α and ER-β were -88.713 and 94.0201. While, score of estradiol with ER-α and ER-β were 94.3455 and -96.1768. Two dimension visualization showed some similar amino acid of binding site between stigamsterol and estradiol *i.e.*, Lys401, Met341, Tyr397, Gly342, and Val338. Hence, stigmasterol in CHEE had potency to compete and interact with estradiol toward estrogen receptor.

Macroscopic Observation and Bone Density Value

Analysis of bone density was done to know the estrogenic effect of corn hair (stigma maydis) ethanolic extract in ovariectomized rats. The deficiency of estrogen is able to result over differentiation of osteoclast cell so that resorptioning the bone excessively. The excessive bone resorption will decreasing the bone density and be one of osteoporosis causes. The macroscopic bone density profile was observed by the magnitude of intensity of white color which is part of the matrix in femur bone epiphysis (Fig. 3). The average of bone density of each treatment group was like below (Fig. 4)

This bone density repairing caused by increasing of estrogen in body that resulted of HCEE administration. Increasing of the estrogen in body can inhibit the osteoclast differentiation The inhibition of differentiation cause bone resorption decreasing and bone density increasing. If the bone density increase so the probability of osteoporosis can be minimalized. The analysis result of bone density is HCEE administration by dose 500 mg/kgBB and 1000 mg/kgBB had not yet increase the ovariectomized rat's femur bone density significantly, compared with control groups OVX without treatment. Thus, it is need to observe microscopically on bone histological profile.

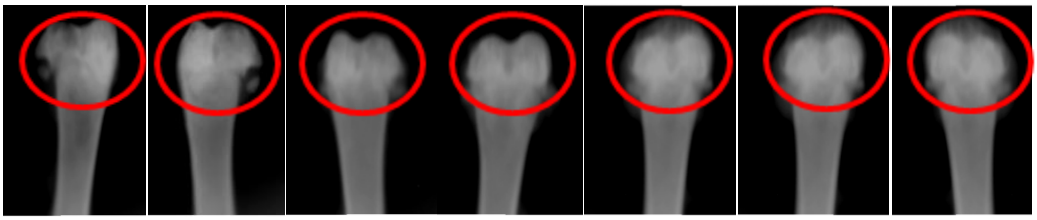


Figure 3. Bone x-ray photos: (a) group 1 (OVX *baseline*); (b) group 2 (NOVX *baseline*); (c) group 3 (OVX+EERJ1000 mg/kgBB); (d) group 4 (OVX+EERJ 500 mg/kgBB); (e) group 5 (OVX+ CMC-Na); (f) group 6 (OVX+17β-estradiol 2μg); (g) group 7 (OVX control without treatment).

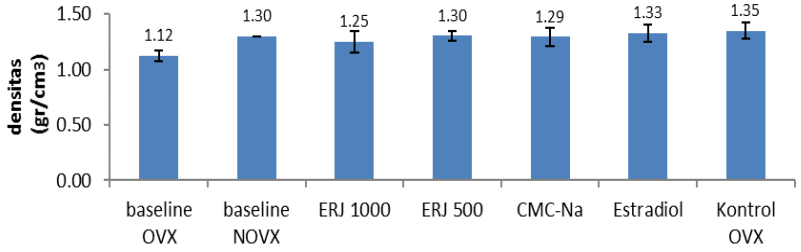


Figure 4. Graphic of the density average of femur bone's treatment group rats. The analysis result of bone density earned either from the average value of density or x-ray photos had not yet showed the result that differ significantly.

Microscopic Observation of Bone Matrix Histological Profile

Rat's demur bone was taken and be made preparation by hematoxylin-eosin (HE) painting. Then observed the widening of canalis haversi on the femur bone histological profile macroscopically. The results of observation showed below (Fig. 5).

The widening of canalis haversi on bone matrix become one of the parameters that show the tendency of bone to leads on osteoporosis process. As wide as canalis haversi show that osteoporosis on the bone higher. In the HE painting result can be looked that there was the size differentiation of canalis haversi between OVX control group and OVS without HCEE administration treatment. The administration of HCEE show that the repair of bone matrix histological profile compared with OVX control group. Thus, the administration of HCEE can repair the ovariectomized rat's femur bone histological profile and the occurrence of osteoporosis can be minimalized.

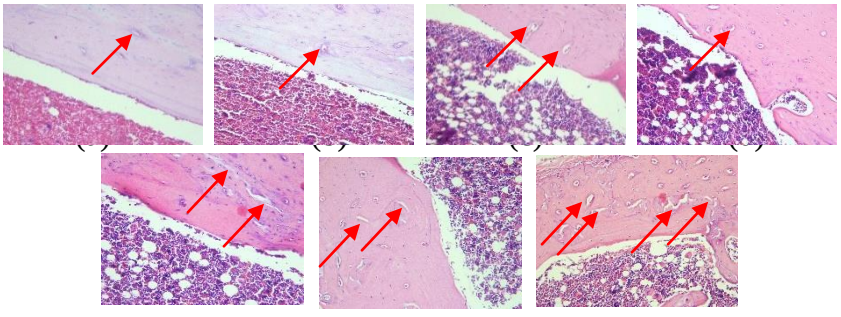


Figure 5. Observation of femur bone histology: (a) group 1 (OVX baseline); (b) group 2 (NOVX baseline); (c) group 3 (OVX+EERJ 1000 mg/kgBB); (d) group 4 (OVX+EERJ 500 mg/kgBB); (e) group 5 (OVX+ CMC-Na); (f) group 6 (OVX+17β-estradiol 2μg); (g) group 7 (OVX control without treatment). The red arrows show The existence of canalis haversi on bone matrix.

CONCLUSION

Corn hair ethanolic extract (HCEE) is able to repair the histological profile of ovariectomized rat bone. HCEE that contain stigmasterol compound is also bonded with estrogen receptor and predicted can compete with estrogen hormone through molecular docking.

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