

# Ethanollic Extract of Papaya (*Carica papaya*) Leaves Improves Blood Cholesterol Profiles and Bone Density in Ovariectomized Rats

Raisatun Nisa Sugiyanto<sup>1</sup>, Rahmi Khamsita<sup>1</sup>, and Ratna Asmah Susidarti<sup>2\*</sup>

<sup>1</sup>Cancer Chemoprevention Research Center Faculty of Pharmacy, Universitas Gadjah Mada, Jalan Sekip Utara 555281 (Phone. 0274 6492662 Fax. 543120) <http://ccrc.farmasi.ugm.ac.id>

<sup>2</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada Jalan Sekip Utara, Yogyakarta 555281, Indonesia

## Abstract

Bone loss and disturbance in the blood cholesterol profiles modulation are two effects caused by menopause syndromes. As the estrogen concentration in the body decreased drastically, menopause women need the replacement of estrogen to keep the regulation of several physiological functions in the body, such as bone generation and cholesterol regulation in a good condition. Phytoestrogen in *Carica papaya* leaves, such as quercetin, could be one of the potential agents for the estrogenic effect. The aim of this study is to know the effects of papaya leaf extract (PLE) on the blood cholesterol profiles and bone density in ovariectomized rats. Thirty six female Sprague Dawley rats divided into six groups. The groups were sham-treated ovx (S-OVX), ovariectomized rats (OVX), CMC-Na control (OVX+CMC-Na), positive control (OVX+Estradiol), and the PLE treatment groups dose 750 mg/kgBW (OVX+750mg/kgBW) and dose 1000 mg/kgBW (OVX+1000 mg/kgBW). Administrations of PLE were done in three weeks orally and estradiol administrated intraperitoneally. In the end of the treatment, the blood sample of tested animals was collected for the blood cholesterol determination (LDL, HDL, triglyceride, and total cholesterol) and the femur bones were examined for the bone density. Based on the results, PLE dose of 750 mg/kgBW a day in ovariectomized rats showed estrogenic effects in modulating blood cholesterol profile by lowering total cholesterol levels. Meanwhile, PLE dose of 1000 mg/kgBW significantly increased the bone density ( $p < 0.05$ ). Thus, PLE is potential to overcome the negative effects of post-menstrual women especially in the cholesterol blood profiles and bone density.

**Keywords:** *Carica papaya*, phytoestrogen, bone density, blood cholesterol, ovariectomized rats

## INTRODUCTION

Recent studies showed that the prevalence of osteoporosis in the world reach 200 million cases, and based on The Indonesian Department of Health, osteoporosis were experienced by around 40 million of population in Indonesia (Kosnayani, 2007). Investigation of the role of dietary habits on the development and prevention of postmenopausal osteoporosis has focused primarily on calcium intake and vitamin D repletion. These two factors explain the development of osteoporosis only in part. Based on Prince and Daper (2000), although the incidence of osteoporosis in homogeneous populations correlates inversely with calcium intake, it appears that the incidence of fractures is still experienced by the populations with high

calcium consumption. Since differences in nutrition between populations can be striking and are not limited to calcium intake, it is possible that other macro- and micronutrients may contribute to the different incidence of fractures between populations.

Phytoestrogen are a large group of heterocyclic phenols with chemical structure similar to estradiol. Phytoestrogen are abundant in plants and have received increasing attention as dietary components that can affect several aspects of human health.

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\*Corresponding author e-mail: [susidarti@yahoo.com](mailto:susidarti@yahoo.com)

The notion that phytoestrogen may affect osteoporosis favorably has emerged recently and is supported by the observations that the phytoestrogen are beneficial for manifestations of the postmenopausal state such as hyper blood cholesterol level and hot flushes. Phytoestrogen also plays a role as estrogen agonist which benefits in the decrease of bone resorption and increase the bone density (Ruggiero *and* Likis, 2002).

Several mechanisms by which phytoestrogens reduce blood cholesterol levels have been proposed. Similar to that of estradiol, first, the cholesterol-lowering effect of phytoestrogens is most likely mediated by its activation of the LDL receptor. By the activation of LDL receptor, the LDL could be metabolized so that LDL level could be decreased (Gruber *et al.*, 2002). Second, phytoestrogens up-regulate the sterol regulatory binding protein 2 (SREBP-2), which in turn produces an increase in surface LDL-receptor expression (Whitten *et al.*, 2004). Third, phytoestrogens decrease blood lipids by their action on peroxisome proliferator activated receptors (PPARs) (Lunden *et al.*, 2007)

A recent study showed that bone lost induced by ovariectomy prevented by feeding rats with diet including phytoestrogen. According to Canini *et al.* (2007), methanolic extract of papaya leaves contain quercetin 0.04 mg/g of 0.25 mg/g of dry leaves. Thus the development of papaya leaves as phytoestrogens through various studies, *in vitro*, *in vivo*, and *in silico* is needed. In this study, *in vivo* experiment was conducted to determine the effects of papaya extract on blood cholesterol profiles and bone density. Hopefully, the results of this study can be used as a reference for further research to utilize papaya leaves extract (PLE) as an alternative source of phytoestrogen.

## MATERIALS AND METHODS

### Materials

Papaya leaves were obtained from Bantul Yogyakarta, ethanol 70% (*E. Merck*), CMC Na (*E. Merck*), aquadest, formaldehyde (Asia lab), NaCl 0.9 % (PT Otsuka, Jakarta),  $\beta$ -estradiol (*Sigma*), corn oil, cholesterol kit standard (DSI, Jerman), *Plain catgut sutures* 3/0 Meiyi® (Huaian Meiyi Medical Instruments Co., Ltd, Jiangsu), antibiotic Enbatic® (PT Erel, Semarang).

### Papaya Leaves Extract (PLE)

Papaya leaves were obtained from Bantul, Yogyakarta. Determination was conducted by Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Gadjah Mada. Papaya leaves were dried and powdered before the extraction process. The extraction was done by ethanol maceration for 5 days. Filtrate collected was concentrated by using rotary evaporator.

### Animals and Treatment

Thirty six of Female Sprague Dawley rats aged 6-7 weeks divided in to 6 groups of treatment. The groups are sham-ovariectomized (S-OVX), control ovariectomy (OVX), ovariectomized+CMC-Na control (OVX+CMC-Na), positive control (OVX+Estradiol), and the PLE treated groups dose 750 mg/kgBW (OVX+750mg/kgBW) and dose 1000 mg/kgBW (OVX+1000 mg/kgBW). After 3 weeks administrations of PLE, the blood was collected from orbital sinus for cholesterol profile analysis and animals were sacrificed. Ovary, uterus, mammae, and bone density were analyzed.

### Analysis of Blood Cholesterol Profiles (LDL, HDL, Triglycerides, and Total Cholesterol)

Blood samples taken from the orbital sinus were incubated at room temperature 15 for minutes and centrifuged at 4000 rpm for 20 min. Serum obtained were used for determination of LDL, HDL, triglycerides, and total cholesterol by enzymatic-colorimetric method. For total cholesterol and triglyceride analysis, as many as 10  $\mu$ L serum were added 1 ml of serum cholesterol reagent solution (or triglyceride reagent solution), and shaken gently allowed to stand at room temperature for 10 minutes and determined for the absorbance at  $\lambda$  546 nm. HDL concentrations were determined by the deposition of lipoproteins in the exception of LDL, VLDL, and chylomicron then continued by enzymatic-colorimetric method. Precipitation reagent used was 0.2 ml of  $Mg^{2+}$  and 0.5 ml of HDL precipitant containing  $MgCl_2$  (25mmol/L) and phosphotungstic acid (0.55 mmol/L). Solution mixture were shaken gently and allowed to stand at room temperature for 10 minutes and then centrifuged at 1200rpm for 2 minutes. The precipitate was then separated from the

supernatant, HDL concentrations were measured with a spectrophotometer containing 0.1 ml of the supernatant was added 1 ml cholesterol reagent then shaken gently and allowed to stand at room temperature for 10 minutes and then determined the absorbance at  $\lambda$  546 nm. For the calculation of the concentration of LDL the formula were used: The concentration of LDL (mg/dL)=total cholesterol concentration-(concentration of triglyceride/5+concentration of HDL). ANOVA one way ( $p < 0.05$ ) were used to analyze the data.

### Analysis of Bone Density

Animal femurs were taken during necropsy and were analyzed by X-ray to observe the qualitative density profile, whereas quantitative analysis is based on the atonation linear coefficient and obtained bone density values ( $\text{g}/\text{cm}^3$ ).

## RESULTS AND DISCUSSION

### Analysis of Blood Cholesterol Profiles (LDL, HDL, Triglycerides, and Total Cholesterol)

Ovariectomy was conducted to provide estrogen deficiency conditions that represent the condition of women in the post-menstrual period (menopause). Total cholesterol, LDL, HDL, and triglycerides were analyzed by enzymatic-colorimetric method to obtain the absorbance value of lipid fractions-serum reagent complex. The results were shown in Fig. 1, OVX experienced a decrease of HDL and an increased of LDL, triglycerides, and total cholesterol. The control estradiol group increased levels of HDL, and lower LDL triglycerides, and total cholesterol levels. Treatment using PLE gave similar results to the control estradiol groups. PLE dose of 1000 mg/KgBW decreased total cholesterol levels significantly ( $p < 0.05$ ).

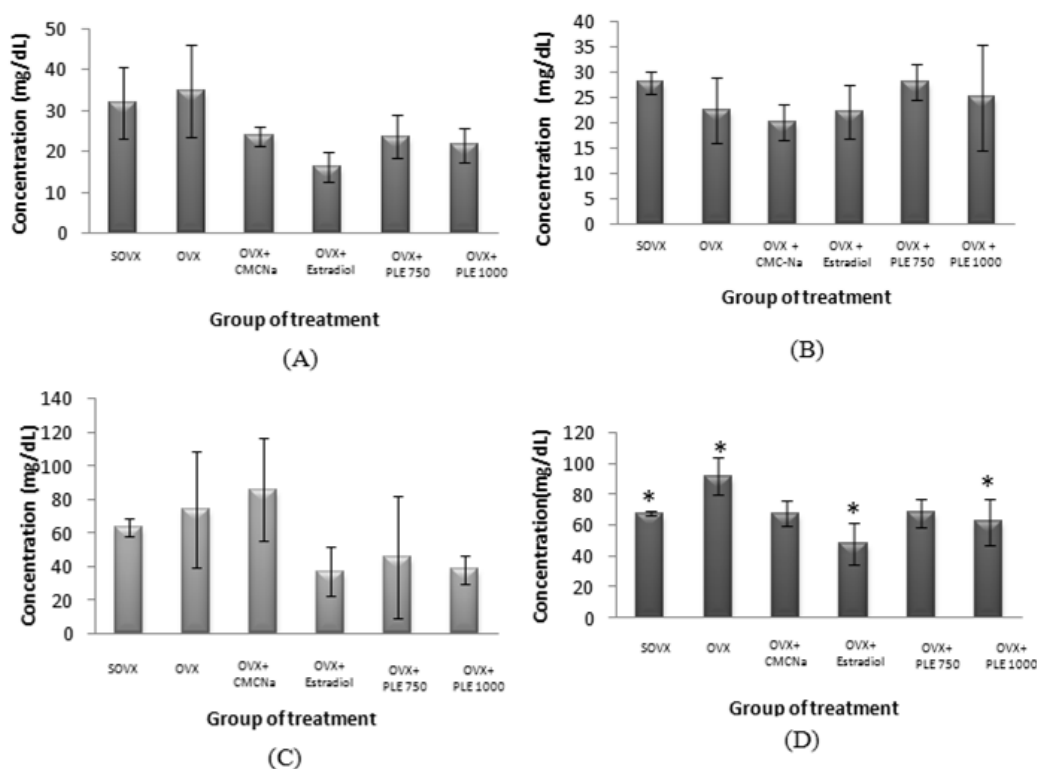


Figure 1. Effect of PLE in blood cholesterol profile. Profile of (A) LDL, (B) HDL, (C) triglycerides, (D) total cholesterol. Note (\*) indicates a significant effect compared to OVX groups. Analysis were done based on one-way ANOVA followed by post-hoc Tuckey HSD ( $p > 0.05$ ).

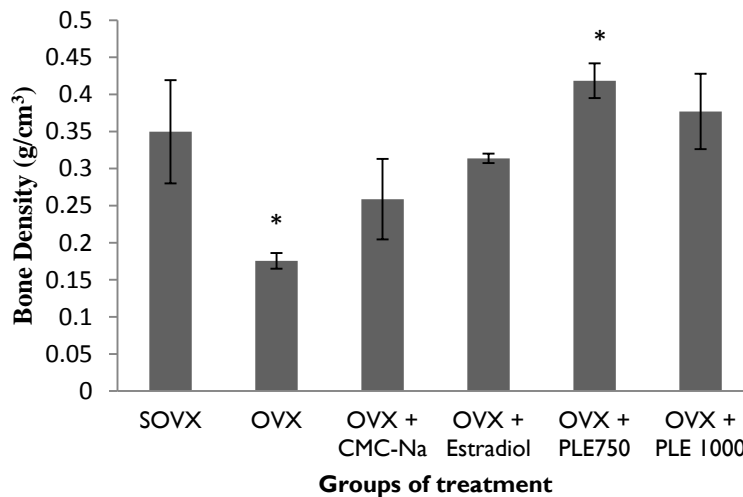
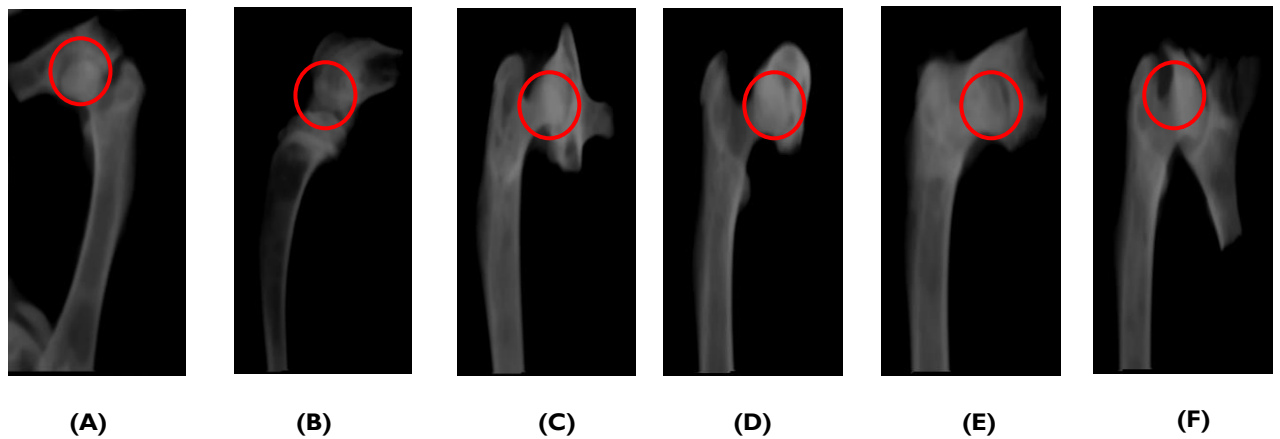
**Table I. The value of blood cholesterol profile by enzymatic colorimetric methods**

Groups	Total cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)
SOVX	67.3 ± 1.48	31.8 ± 14.2	27.9 ± 4.40	63.1 ± 5.67
OVX	91.6 ± 10.4	34.6 ± 8.87	22.3 ± 8.30	74.2 ± 31.03
OVX+CMC Na	67.7 ± 7.67	23.6 ± 0.76	20.0 ± 1.35	85.7 ± 23.28
OVX+Estradiol	47.8 ± 4.09	16.2 ± 0.95	22.2 ± 3.65	37.1 ± 8.06
OVX+PLE 750 mg/KgBW	68.1 ± 8.6	23.6 ± 5.25	28.0 ± 2.50	45.5 ± 18.68
OVX+PLE 1000 mg/KgBW	62 ± 12	21.6 ± 3.48	24.9 ± 4.60	38.3 ± 2.71

**Analysis of Bone Density**

The analysis of bone density was provided to know the effects of PLE in estrogen deficiency condition by ovariectomy. From the results using X-rays (Fig.2 and Table II), bone

density were decreased in ovariectomized group, and increased in control estradiol groups and also on the treatment of PLE dose 750 and 1000 mg/kgBW.



(G)

**Figure 2. Effects of PLE on bone density. The results of X-ray bone of (A) Sham-OVX rat group, (B) OVX, (C) OVX+CMC-Na, (D) OVX+estradiol, (E) OVX+PLE 750 mg/kg, (F) OVX+PLE 1000 mg/kg. Red circle sign indicates the condition of the bone density of rat per treatment. (G) Average density bone of each group. Note (\*) indicates a significant effect compared to OVX groups. Analysis were done using on one-way ANOVA followed by post-hoc Tuckey HSD (p>0.005).**

**Table II. The bone density value**

Groups	Density (g/cm <sup>3</sup> )
SOVX	0.3497 ± 0.12
OVX	0.1756 ± 0.02
OVX+CMC Na	0.2587 ± 0.09
OVX+Estradiol	0.3137 ± 0.01
OVX+PLE 750 mg/KgBW	0.4184 ± 0.04
OVX+PLE 1000 mg/KgBW	0.3769 ± 0.08

It is shown that the bone density profile of the ovx-treated groups (OVX) was lower than the control sham-ovariectomy (SOVX) significantly which related to bone desorption in estrogen deficiency condition. Estradiol treatment (OVX+estradiol), increased the bone density. The treatment of PLE increased the bone density, especially at dose 750mg/kgBW. These results showed that the treatment of PLE improved bone loss in the estrogen deficiency condition. The mechanism of how PLE increasing bone density is need to be further investigated, but it might be the same as the mechanism of estrogen in modulating bone density.

In the post-menstrual period, women experience estrogen deficiency conditions that lead to various changes in the body. The menopause women tend to experience the abnormalities change in lipid metabolism. Disorder of lipid metabolism was characterized by increase or decrease in plasma lipid fractions. The abnormalities mostly happened are the increase in total cholesterol, LDL cholesterol, increases in triglyceride levels and decrease HDL.

Estrogen could interact with estrogen receptor and influence the formation of HDL and LDL receptor so the HDL could be increased while LDL is decreased. The increased of HDL blood level caused by expression of apoA-1 protein which is one of the substances important in the formation of HDL. Higher expression of apoA-1 increases the levels of HDL in the blood (Harnish *et al.*, 1998). Furthermore, increase levels of HDL in the blood will increase the transport of extra hepatic LDL to be excreted through the urine. Estrogen also play role in LDL level since estrogen modulates LDL receptor transcription. LDL receptor binds to LDL in the blood through the endocytosis mechanism resulting in LDL metabolism. Then, LDL is metabolized and will be broken down in the liver lysosomes (Gent *et al.*, 2004). The metabolism includes the role of apolipoprotein and cholesterol ester

hydrolysis and also cholesterol translocation into the cell (Murray *et al.*, 1995). Both of the above mechanisms reinforce the reason that estrogen can modulate the levels of HDL and LDL in the blood.

In the mechanism of estrogen modulating bone density, estrogen affects bone remodeling on trabecular and endocortical (Prince and Draper, 2000). Estrogen affects the activity of osteoblasts and osteoclasts, including maintaining the balance of those cells through the regulation of the production of paracrine factors. Osteoblast cells have estrogen receptors ER $\alpha$  and ER $\beta$  in the cytosol, thus estrogen affects mainly in osteoblast regulation. In the differentiation of osteoblast, cellsexpress ER $\alpha$  10 times than ER $\beta$  (Monroe *et al.*, 2003). Under the normal circumstances, circulating estrogen in the body will reach osteoblasts, and interacts with the estrogen receptors located in the cytosol of the cells. Then, this kind of mechanism results in the decrease of cytokines secretion, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ), which functions in bone resorption. On the other hand, estrogen increases the secretion of transforming growth factor  $\beta$  (TGF- $\beta$ ), which is the only growth factor as the mediator to attract osteoblasts to the bone hole caused by osteoclasts activity. Thus, osteoblast cells are the primary target cells of estrogen to release several growth factors and cytokines as described above, and indirectly or directly also affect osteoclasts (Monroe *et al.*, 2003).

Conditions of estrogen deficiency lead to osteoclastogenesis and bone loss. However, the administration of estrogen occurring bone remodeling, and decreased production of IL-1, IL-6, and TNF- $\alpha$ , as well as the of macrophage-colony stimulating factor (M-CSF) and RANK-Ligand (RANK-L). Meanwhile, the estrogen also stimulates the expression of osteoprotegerin (OPG) and TGF- $\beta$  (Transforming Growth Factor- $\beta$ ) in osteoblasts and stromal cells, which would further inhibit

bone resorption and increases apoptosis of osteoclast cells (Monroe *et al.*, 2003). The results of this study concluded that PLE could be a source of phytoestrogens which provide estrogenic effects in the cholesterol modulation and improved bone density profiles.

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