Estrogenic Activity of Ethanolic Extract of Papaya Peels (Carica Papaya L.) on Uterine Weight And Mammae Gland Proliferation on Ovariectomy Rats

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Abstract

Papaya bark is one of Indonesia’s natural wealth that contains flavonoid compounds such as myricetin and kaempferol that included in the phytoestrogen compounds. The aim of this study is to examine the estrogenic effects of ethanolic extract of papaya peels (EEPP) on the development of mammae gland and the increasing of uterine weight. The in vivo test was performed in ovariectomized Sprague Dawley female rats. After 30 days of treatment, animals were sacrificed to take the uterus and mammae glands. Measurement of uterine weight and mammae gland was observed by hematoxylin-eosin staining method to know the lobulus development and AgNOR staining to determine the proliferation level of mammae gland epithelial cells. The results showed that EEPP at the concentrations of 500 and 1000 mg/kgBW were able to increase uterine weight and proliferation of mammae gland. In conclusion, papaya bark has the potential as phytoestrogen compound to maintain female reproductive health and woman beauty.

Keywords: Ethanolic extract of papaya peels (EEPP), phytoestrogen, ovariectomized rats, uterine weight, mammae proliferation

INTRODUCTION

Post menstrual or menopause is a natural process that will be experienced by every woman that marked by a significant decrease in estrogen hormone. The estrogen hormone is known to be responsible for secondary development in women’s bodies such as breast and uterus (Frasor, et al., 2003). Deficiency in estrogen can lead to discomfort and decrease in the quality of life of women such as hot flashes, insomnia, sexual dysfunction, vaginal dryness, cardiovascular disease, and bone loss (Mendelsohn and Karas, 2010). The discomfort can be overcome by giving estrogen from outside the body known as Hormone Replacement Therapy (HRT). However, long-term use of HRT has many side effects and increases the incidence of breast cancer (Chlebowski, et al., 2009). Therefore, a safe
potential alternative treatment is needed, one of them is by using phytoestrogens. Phytoestrogens are chemical compounds in plants that have estrogenic activity that can replace the function of estrogen (Yildiz, 2005).

One of the natural ingredients in Indonesia that reported to have phytoestrogen content is papaya peels (Carica papaya L.) which in everyday is still rarely used. The compounds contained in the peels of papaya are myricetin and kaempferol that belong to the flavonoid group (Rivera-Pastrana, et al., 2010). Myricetin and kaempferol have been shown to have an estrogenic effect on in vitro assays (Maggiolini, et al., 2005, Oh, et al., 2006) and in vivo tests according to Pratama, et al. (2011) and Trivedi, et al. (2008).

This study aims to determine the interaction of flavonoid myricetin and kaempferol compounds contained in EEPP to estrogen receptor through molecular docking. Then became the basic of in vivo testing to determine the effects of EEPP on the development of ovariectomized Sprague Dawley rat female mamma gland. The results of this study are expected to be the basic in solving the problem of beauty and reproductive health in menopausal women.

The aim of this study is to investigate the estrogenic effect of papaya peels on uterine weight and proliferation of mammae gland in ovariectomized Sprague Dawley female rats.

MATERIALS AND METHODS

Tools and Materials

Papaya peels obtained from Wedomartani, Sleman, Yogyakarta, Indonesia. 70% ethanol (Merck), ketamine 100 mg/mL, plain catgut sutures 3/0 Meiyi®, NaCl 0.9% (PT Otsuka, Jakarta), Enbatic® antibiotic (PT Erela, Semarang), Betadin® (PT Mahakam Beta Farma, Jakarta), CMC-Na (E.Merck), aquades, 10% formalin (Asia lab.), Estradiol (Sigma), Haematoxylin-Eosin staining reagents and AgNO3.

Extraction of Papaya Peels

Papaya peels obtained from Wedomartani, Sleman. Accumulated papaya peels is dried with oven, then made powder using blender and extracted with 70% ethanol (maceration). The obtained maserate was concentrated using a rotary evaporator into a viscous extract.

in vivo Assay

Thirty five of Sprague Dawley female rats were placed in a plastic cage with husk pads and fed with pellets and given drink of PDAM water (ad libitum). The extract was given in the form of a suspension in a 0.5% CMC-Na solution. The extract solution was made freshly before it was administered to the test animals. Rats were divided into seven treatment groups with 5 rats per group: Group I: non-ovariectomized base line (NOVX), Group II: ovariectomy base line (OVX), Group III: ovariectomy control, Group IV: 0.5% CMC-Na treatment (control negative), Group V: treatment of estradiol 2 μg/day, Group VI: treatment of EEPP concentration of 500 mg/kgBW, Group VII: treatment of EEPP concentration of 1000 mg/kgBW. Group II to VII were excised at age 70 days. For group I, rats were conditioned as if they were ovariectomized. The experiment was carried out for 1 month, at the end of the experiment all rats uterine were taken and mammae glands to be analyzed.

Determination of Uterine Weight

Wet uterine samples are taken at the end of the treatment period. The cleansed sample was weighed using the analytical balance and the weight being recorded.

Haematoxylin-Eosin (HE) Staining

Mamme glands were sliced, then made in paraffin blocks and cut thinly with 4 μm thick, then stained with hematoxylin dye and followed by eosin, then mounted and covered with a glass deck. Qualitative observations were performed under a light microscope with 40x magnification, whereas...
quantitative tests were used to calculate the number of lobolus cells using 3 different fields of view.

**Staining of Agryrophyllic Nucleolar Organizer Region (AgNOR)**

Histologic tissue preparate were prepared in the buffer then incubated in the autoclave. The preparate then stained with silver staining mixture, dehydrated with ethanol and affixed to the resin or synthesis medium. Observations were made by counting black dots converted to mAgNOR values as the average number of black dots on at least 100 cells.

**Data Analysis**

The results of uterine weight were analyzed by MS Office Excel 2010 in units of mg/100g BW. The statistical test was performed with SPSS version 17.0 in the Kolmogorov-Smirnov test to determine the normality of the data. Statistical significance test for normal distributed data is performed with one way ANOVA followed by post-hoc Tukey HSD test with 95% confidence level. The results of H&E and AgNOR staining were statistically analyzed by one-way ANOVA and post hoc Tukey HSD test at 95% confidence level.

**RESULTS AND DISCUSSION**

**Effect of EEPP in Increasing Uterus Weight of Ovariectomized Rat**

Examination of uterine weights was performed to determine the effect of EEPP on uterus animal weights (Table 1.)

Table 1 shows the differences in uterine weight between the baseline group of NOVX and OVX, indicating that the ovariectomy operation was successful. The ability of EEPP as phytoestrogen is shown from the comparison of uterine weight of the test group with the untreated group. From these results, EEPP has a tendency to increase uterine weight in female Sprague Dawley ovariectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Uterine weight (mg/100gr BW)</th>
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</thead>
<tbody>
<tr>
<td>OVX Baseline</td>
<td>56.67 ± 29.14</td>
</tr>
<tr>
<td>NOVX Baseline</td>
<td>398.22 ± 98.64</td>
</tr>
<tr>
<td>EEPP 1000 mg/kgBW</td>
<td>279.36 ± 123.40</td>
</tr>
<tr>
<td>EEPP 500 mg/kgBW</td>
<td>277.59 ± 262.66</td>
</tr>
<tr>
<td>CMC-Na</td>
<td>28.05 ± 300.50</td>
</tr>
<tr>
<td>Estradiol</td>
<td>142.15 ± 289.15</td>
</tr>
<tr>
<td>Untreated</td>
<td>36.89 ± 160.99</td>
</tr>
</tbody>
</table>

Data analysis using one way ANOVA test followed by Tukey HSD test with 95% confidence level.

Estrogen plays an important role in uterine development. At puberty the uterus has a high response to estrogen. The presence of estrogen stimulates the increase in water content in cells, DNA, RNA, protein synthesis, and activation of enzymes in the uterus. This event results in an increase in uterine weight due to the entry of water into the tissue of the uterus followed by cell proliferation and the accumulation of solids in the uterus (Turner and Bagnara, 1988). Further research suggests that in the uterus, the bond between estrogen and the ERα receptor causes proliferation of uterine cells, luminal epithelium and uterine estrogen receptor genes that impact on uterine weight increased (Frasor, et al., 2003).

**The Effect of Haematoxyllin and Eosin (H&E) Staining**

Staining is done to see histologic images of breast cells from the test animals. The estrogenic effects of EEPP were seen from microscopic lobular images. Then the number of lobules is quantified by observing each preparate of three planes view. From Figure 1, it can be seen that the administration of EEPP concentration of 500 mg/kgBW was able to increase the number of lobular cells when compared with the OVX control group, but at a concentration of 1000 mg/kgBW was not able to increase the number of lobular cells compared with 500 mg/
Figure 1. Histologic picture of rats mammae gland with HE painting after EEPP treatment. Mammae glands were observed with HE staining. (a) The baseline nonovariectomy group (NOVX), (b) the baseline ovariectomy group (OVX), (c) the OVX + CMC-Na group, (d) the OVX + estradiol group 2 μg/day group, (e) the OVX + EEPP 500 mg/kgBW group, (f) OVX + EEPP 1000 mg/kgBW group, and (g) OVX control group. Observations were performed under a light microscope with a magnification of 40x. The arrows show the lymph nodes of mammae gland. EEPP estrogenic effects are indicated by an increase in the number of lobules compared with the control group.

kgBW concentration. From these results, EEPP tends to increase the development of mammae gland by increasing breast lobule cells (Figure 2).

Effect of EEPP on Agyrophyllic Nucleolar Organizer Region (AgNOR) Staining

Staining AgNOR (Agyrophilic Nucleolar Organizer Region) or silver staining is performed to determine the proliferative effect of EEPP on breast cells in ovariectomized rats. This method is able to show cell division by quantifying the Nucleolar Organizer Region (NOR) which indicates cell proliferation activity (Derenzini et al., 2003). The calculated number of black dots is quantified in mAgNOR by counting the total number of black dots on at least 100 cells then on average by dividing the total number of black dots. From the picture above, seen in the provision of EEPP concentration of 500 mg/kgBW can increase the number of black dots more than 1000 mg/kgBW

Figure 2. Number of lobules in the ovariectomized rats graph. Amount of lobules are quantified by observing the prepartate in three different fields of view. The displayed image shows the mean ± SE value of 3 experiments. Data analysis using one way ANOVA test followed by Tukey HSD test with 95% confidence level.
concentration. From these results, EEPP tends to increase the development of mammary gland with the increased of breast cells proliferation (Figure 3, Figure 4).

CONCLUSION

Based on in vivo studies that have been done, it can be concluded that EEPP can be used as a source of phytoestrogens that have been prove to increase the uterine weight and increase the proliferation of mammary gland cells.

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