

The Utilization of *Graptophyllum pictum* (L) Griff. Extract as the Endometrial and Myometrial Hyperplasia Inhibitor of Ovariectomized Mice

Listijani Suhargo and Dwi Winarni

Department of Biology, FST, Airlangga University, Surabaya

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Abstract: Endometrial and myometrial hyperplasia was caused as a side effect of estradiol hormone using for menopausal condition and it could change to be cancer. *Graptophyllum pictum* (L) Griff. (Daun wungu) ethanol extract with its flavonoid competes with estradiol to bind the estrogen reseptor and decreases the side effect of estradiol. The aim of this research was conducted to evaluate the utilization of daun wungu ethanol extracts as the endometrial and myometrial hyperplasia inhibitor of ovariectomy mice. This researches used 30 ovariectomized mice. The ovariectomized mice were grouped in 5 groups, one group control group with 3 µg/kg ethynil estradiol/EE), 4 groups for the treatment with 3 µg/kg EE with 25 mg/kg, 37,5 mg/kg, 50 mg/kg and 62,5 mg/kg daun wungu extracts in coconut oil. All treatments were done by oral for 30 days. At the end of the treatments, all mice was sacrificed and were made histology preparation of uterus. The result showed that daun wungu ethanol extracts could inhibit endometrium and myometrium hyperplasia that were showed in the thickness of those tissues of ovariectomized mice. And 37,5 mg/kg daun wungu extract was optimal dose for hyperplasia inhibitor of those tissues.

1 INTRODUCTION

In general, the female reproductive cycle stops at the age of 45 to 50 years due to the absence of a growing follicle. That condition is called menopausal condition. At this time the estrogen hormone is not produced by follicles in the ovary. Decreased levels of estrogen cause several symptoms such as heat fluctuation, heart palpitations, sleep disorders, irritability, headache, tingling, libido disorders, obstipation and weight gain (Burger, 2007). Not all postmenopausal women experience menopausal symptoms because estrogen is also produced in fatty and muscle tissues through aromatization of adrostenedione. Androstenedione is produced by the adrenal glands (95%) and ovaries (5%) (Simpson, 2002).

In menopausal women the presence of estrogen produced by muscle and fat cells can also affect the growth of endometrial and myometrial cells. The growth of these cells might increase and was called hyperplasia in overweight women or who use estrogen therapy during menopause. Estrogen therapy using synthetic estrogens such as diethyl

stilbestrol, hexestrol, dienestrol and ethynil estradiol could increase cell proliferation through synthetic estrogen binding to estrogen receptors. Increased cell proliferation or cell hyperplasia might lead to change in cell genetic properties and may further lead to the occurrence of cancer cells (Pinkerton, 2017).

The use of natural ingredients to suppress the effects of estrogen needs to be investigated with the aim of inhibiting the hyperplasia of uterine cells. One of the natural ingredients is daun wungu (*Graptophyllum pictum* L. Griff.) which is known to contain flavonoids and phytosterols (Isnawati, 2003). Flavonoids contained in daun wungu are myricetin and kaempferol (Kusumawati et al., 2002).

The study was aimed to find out the role of daun wungu (*Graptophyllum pictum* L. Griff.) as inhibitors of endometrial and myometrial cell proliferation in ovariectomized mice. Ovariectomized mice are model animals of menopausal conditions. Table 1 shew the mean data of endometrial thickness and myometrium of ovariectomy mice with daun wungu and EE (Ethynil estradiol) treatment.

Tabel 1. The effect of daun wungu (*Graptophyllum pictum* L.Griff.) extract on endometrial and myometrial thickness of ovariectomized mice (μm)

The measurement of	The treatment for ovariectomized mice with 3 $\mu\text{g}/\text{kg}$ EE plus daun wungu extract with doses				
	Control (EE)	25 mg/kg	37,5mg/kg	50 mg/kg	62,5mg/kg
Endometrial thickness	441,9 \pm 59,8	397,9 \pm 30,1	297,7 \pm 85,7	422,1 \pm 54,2	411,9 \pm 72,3
Myometrial thickness	63,6 \pm 8,6	69,3 \pm 9,6	50,2 \pm 8,3	58,7 \pm 8,8	59,9 \pm 10,1

The results of the research indicated that the ethanol extract of daun wungu (*Graptophyllum pictum* L. Griff.) had a maximum role as endometrial and myometrial cell proliferation inhibitor at 37,5 mg/kg dose. Ovariectomized mice as experimental animals were given ethinyl estradiol for increased proliferation of epithelial cells, fibroblast and endothelial cells. Flavonoid of daun wungu with kaempferol and myricetin content had chemical structures that resemble estradiol so they could bind to estrogen receptors. Flavonoids

competed with estradiol to bind estrogen receptors. The estrogenic effect of flavonoid was lower than EE to induce cell proliferation on endometrium and myometrium, so daun wungu extract could reduce the high estrogenic effects of EE. Flavonoid of daun wungu also can inhibit aromatase activity that plays a role to alter androgens to estrogen and further decrease the effect of cells proliferation on endometrial and myometrial cells (Suggest *et al.*, 2014 and Guo *et al.*, 2012).

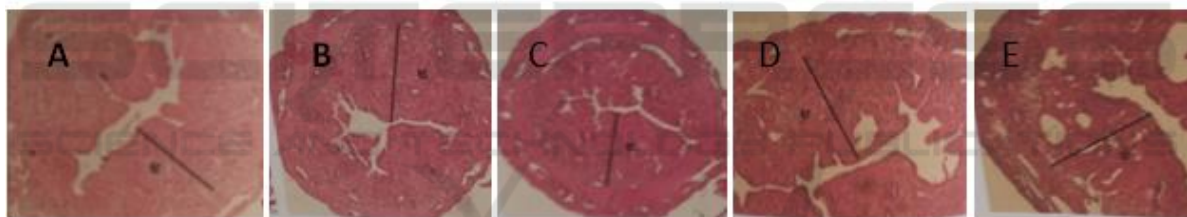


Figure 1. Uterus section of ovariectomy mice with 5 treatment. A. Control (EE). B.C.D.E.treatment with EE and daun wungu extract (25 mg/kg, 37,5mg/kg, 50mg/kg and 62,5mg/kg)

Daun Wungu contains phytosterols that plays a role to inhibit cholesterol absorption and increase its excretion so as to reduce total cholesterol (Fernandez *et al.*, 2002). Cholesterol is the main ingredient for estrogen formation. Cholesterol is transferred to the mitochondria of the adrenal gland cells and converted to pregnenolone. Pregnenolone was transferred to the cytoplasm and formed progesterone and then became androstenedione (Miller, 2005). Androstenedione leads to the endometrial cells through the blood vessels and was converted by aromatase enzyme to estrone and then became estradiol. Estradiol increased proliferation of endometrial and myometrial cells. The phytosterol content of daun wungu extract would decrease the synthesis of estradiol (Simpson, 2002).

At the lowest dose 25 mg/kg of daun wungu extract could not inhibit cell proliferation due to dominant induction of ethinyl estradiol and less flavonoid of daun wungu extract couldn't compete with ethinyl estradiol in β -estrogen receptor. In higher doses 50 mg/kg and 62.5 mg / kg of daun wungu extract also couldn't inhibit cell proliferation, it was caused that higher doses of flavonoid and fitosterol bound to β -estrogen receptor could increase the proliferation of endometrial and myometrial cells in addition to the effects of Ethinyl estradiol, consequently the inhibitory effect of proliferation cells are insignificant and cell proliferation still increased.

2 Experimental

2.1 Daun Wungu Extraction Procedure

Daun Wungu were weighed as much as 1 kg, it were dried in oven to remove water content with temperature 40°C, then were mashed with a blender to form a leaf powder. Daun wungu powder was macerated with 90% ethanol, the filtration was evaporated with rotary evaporator for 48 hours. The results obtained are ethanol extract of daun wungu (Setiawan, 2012).

2.2 Ovariectomy Via Ventral Route

Anesthetize mice with 0.5 cc ketalar 10 mg / bw via intramuscular injection on femur, the mice were stretched and feathered was wetted by ethanol 70% around the midline of the lower abdomen. and made incision in the middle as long as 1 cm for skin, muscle and peritoneal layer. Ovarium was removed and binding of fallopian tubes and ovarian cutting. The slices of the peritoneum were stitched, then the muscle and skin sections were stitched. (Miep et al., 2007).

2.3 Animal Treatment

In this research, 50 mice were used as control group, one group was treated with 3 µg/kg ethinyl estradiol (EE), and 4 other groups were given 3 µg/kg EE and daun wungu extract treatment with dose 25 mg/kg, 37.5 mg/kg, 50 mg/kg and 62.5 mg/kg with coconut oil as solvent. All the treatment were done by oral, each mouse was given 0,1 ml EE and daun wungu extract for 30 days.

2.4 Histology procedure

Uterus were quickly removed at sacrifice and fixed in Bouin Solution. After fixation, tissues were dehydrated in 70%, 80%, 96% ethanol and , then embedded in paraffin and cut on a rotation microtome in 5-6 µm sections, and then stained with hematoxylin & eosin (Hamouda et al., 2018). The endometrial and myometrial thickness were measured with micrometer in microscope.

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