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The Influence of purple sweet potato toward Fas Epitel of White Rat

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Abstract. This study builds on proving the purple sweet potato anthocyanin can decrease the epitel Fas cell of ovariectomy of white rat expression. The research design used was *true* experimental with Randomized post only control group design. The trial animal was female white rat (Rattus norvegicus) aged 9-12 weeks, weight 150-250 gram namely 30 divided into 5 groups: negative control group, positive control group and t giving group with the dose 20 mg/kg weight (A1), dose 40 mg/kg weight (A2), dose 80 mg/kg weight (A3). It is done by ovariectomy during 28 post ovariectomy then giving purple sweet potato anthocyanin during 30 days. The expression of Fas was examined by using IHC. To increase the reliability of the data was analyzed with Independent Sample T Test dan One Way Annova. The result shows that the purple sweet potato anthocyanin can decrease he Fas expression to endometrium cell epitel with the dose 40 mg/kg weight and 80 mg/kg weight significantly. It is caused the purple sweet potato anthocyanin can decrease dead cell of extrinsic. The dead cell was passed by the extrinsic ways. The finding has implication for can decrease the Fas expression of ovariectomy female white rat.

Keywords **:** endometrium epitel cell, fas expression,, purple sweet potato anthocyanin white rat.

I. INTRODUCTION

Menopause is the finishing menstrual period for women forever, while the women got the menstrual period regularly every month. (1).Menopause age between 45-55 years (2) . During menopause, the hormonal changes happen namely the decrease of estrogen hormone in critic value. (3). Estrogen is strong antioxidant whose giving protection toward the oxidative stress during reproductive period. (4). Oxidative is the main point of aging process (5). During the process of oxidative, the DNA damage, per oxidative lipid, and per oxidative protein. It made the mitochondria protein exertion and release into cytoplasm. The release of mitochondria protein into cytoplasm can initiate the apoptosis program happen. (6) Apoptosis of menopause endometrium can happen in intrinsic pathway and extrinsic pathway. Apoptosis in the menopausal endometrium can occur through intrinsic pathways or extrinsic pathways.) In apoptosis through the extrinsic pathway in the endometrium of rats which are ovariectomized it is characterized by an increase in TNFα and Fas. TNFα is a cytokine that acts as a death receptor associated with Fas protein. Fas is tied to its ligand (FasL). Three or more Fas molecules combine

to form FADD (Fas-associated death domain). FADD is attached to the death receptor and begins to bind to the inactive form of caspase 8. The procaspase 8 molecule then breaks into active caspase 8. This enzyme then activates the executor caspase so that apoptosis occurs in ovariectomy rat endometrial epithelial cells (7). According to research conducted by Mor (2001) the withdrawal of the estrogen hormone results in an increase in Fas expression in the endometrium (8). Other studies have shown an increase in the expression of Fas in the endometrium depending on which time it increases by 15% after 3 hours of estrogen hormone withdrawal, 15% after 6 hours of withdrawal, 27% after 24 hours of withdrawal and 200% after 48 hours of withdrawal (9).

One way to reduce oxidative stress is by giving antioxidants. One of the bioactive ingredients that can be used as an antioxidant in reducing oxidative stress is anthocyanin (10). Anthocyanin is a type of flavonoid group polyphenols which gives red, purple and blue colors to fruits, vegetables, flowers and seeds (11). The antioxidants contained in anthocyanin purple sweet potato are higher than red cabbage, grape skin, elderberry, and purple corn (12). Purple sweet potato was found to be 10 times higher in activity of oxygen radical absorbance capacity (ORAC) compared to white, yellow and orange sweet potatoes.

At menopause because there is a decrease in the hormone estrogen resulting in decreased cell proliferation and the process of cell death (apoptosis) increases resulting in endometrial atrophy causing bleeding in menopausal women (4). One of the triggers or triggers of the apoptosis program through extrinsic pathways is Fas protein which will bind to its ligand which will then initiate the apoptosis program. If Fas does not bind to the ligand and does not form a trimer, the apoptosis program does not occur. In the study of ovariectomy rats showed that there was an increase in Fas expression in endometrial epithelial cells. Therefore this study aims to prove the effect of purple sweet potato anthocyanin on decreasing the expression of Fas ovariectomy endometrial epithelial cells.

II. LITERATURE REVIEW

The imbalance of levels of free radicals and antioxidants in the body will cause oxidative stress. During menopause there is a decrease in the hormone estrogen which is a powerful antioxidant in the body (3,13). Estrogen can reduce oxidative stress by modulation of the expression and function of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and antioxidant enzymes (SOD, GPx, and catalase), providing protection against oxidative stress during the reproductive stage. In addition, other studies have shown that estrogen is a powerful antioxidant that can reduce low-density lipoprotein oxidation in vitro and in vivo (14). In addition, the chemical structure of estrogen molecules has antioxidant abilities because of the presence of OH in C3 from the phenolic ring in position A which acts as a free radical catcher that prevents oxidative damage (14). Other studies have shown that a decrease in the estrogen hormone increases oxidative stress depending on the concentration and chemical structure of this hormone. At high concentrations, estrogen tends to have a beneficial antioxidant effect by inhibiting the base 8-hydroxylation of guanine DNA. However, at low concentrations, this hormone has a pro-oxidant effect especially when its chemical structure contains catechol a. Oxidant effects include alkaline oxidation, formation of DNA additives and affecting genetic material (15). In the process of oxidative stress DNA damage occurs which will trigger the apoptosis program (6). Apoptosis is programmed cell death which has an important role in the development and maintenance of health in cellular organisms. This dead cell is a response to various stimuli. During apoptosis, these cells will be controlled and regulated which are then cell phagocytes by macrophages (16). Fas is a stable protein apoptosis marker in female endometrium. Fas and FasL expression is found in human endometrium throughout the menstrual cycle. Fas and Fas L are in the Golgi apparatus and cytoplasmic vesicles during the end of the proliferation phase. Whereas in the secretion phase this protein is expressed in the plasma membrane of endometrial cells where in this phase Fas can bind FasL and apoptotic signals. In the proliferation phase with the presence of estrogen hormone, endometrial cells become resistant to apoptosis (8). With the influence of estrogen endometrial cells proliferate because estrogen induces activation of the antiapoptotic gene and inhibits the proapoptotic gene (17). Withdrawal of estrogen and progesterone hormones in endometrial cells will activate the Fas pathway and induce cell death in endometrial cells in vitro. The role of estrogen and progesterone in Fas and FasL expression is a form of balance between cell survival and cell death where these two hormones play a role in regulation of apoptosis. (8) The mechanism of extrinsic cell apoptosis involves the role of death ligand and death receptors on the cell surface. Ligands that bind to death receptors located on the transmembrane will form a trimer called FADD (Fas Associated Death Domain). The complex formed between the ligand-receptor and FADD is called DISC (Death Inducing Signaling Complex) which will activate caspase 8. Caspase 8 then activates caspase 3 to execute cells to occur apoptosis. The mechanism of intrinsic cell apoptosis involves the role of mitochondria, due to changes in toxic mitochondria which cause the formation of apoptosome which subsequently initiates caspase 9. This Caspase 9 then activates caspase 3 to initiate apoptosis (18). The mechanism of apoptosis through intrinsic and extrinsic pathways can be seen in the scheme below.

Oxidative stress can be lowered by giving antioxidants extracted from plants in the tropics. One of the antioxidants that can be used is anthocyanin. Anthocyanin is a flavonoid group polyphenol which has a structure and biosynthetic path similar to other flavonoid groups, namely is flavones and flavonoids (19). Anthocyanin is found in fruits, vegetables, flowers and seeds such as blueberries, cherries, raspberries, strawberries, black currants, purple grapes, grapes and purple sweet potatoes (11,20). Purple sweet potato has an attractive purple red color, high anthocyanin content, total phenolic and antioxidant activity (21). Purple sweet potatoes contain phenolic compounds which are powerful antioxidants, and have various biological functions such as anti-bacterial, antihistamine, free and ant mutagenic radical catchers (22). The purple sweet potato phenolic content is 261.4 - 712.8 mg per 100 g of dry weight, higher than the phenolic content of yellow sweet potatoes, carrots and carrots (21). Anthocyanin compounds in purple sweet potatoes, especially peonidins and cyanidins have high antioxidant properties and are anti-inflammatory, antihypertensive, atherosclerosis especially when passing through the digestive tract (23). Some studies show that purple sweet potato extract which is rich in anthocyanins, besides being a strong antioxidant, also has antimutagenic activity, can reduce blood pressure and reduce liver injury in mice (21).

III. METHOD

This study uses pure experimental design (true experimental) with a posttest only control group design method. This research was carried out by giving treatment to experimental animals namely white mice (Rattus norvegicus). The white rats in this study were divided into 5 treatment groups, namely: (1) Negative control group (KN), which was a group of white rats that were not ovariectomy and without treatment, (2) Positive control group (KP), which was ovariectomized and without given treatment, (3) Antocyanin I Treatment Group (KA1), namely groups of white rats which were ovariectomized and given anthocyanin 20 mg / kgBB, (4) Anthocyanin II Treatment Group (KA2), which was a group of white rats which were ovariectomized and anthocyanin 40 mg / kg BB, (5) Anthocyanin III Treatment Group (KA3): a group of white rats which were ovariectomized and given anthocyanin 80 mg / kg BW.

This research was conducted in the Pharmacology laboratory of the Medical Faculty of Brawijaya University as a place for animal testing and ovariectomy. Anatomical Pathology laboratory is a place to cut endometrial organs and examination of endometrial thickness, Faal laboratory is an anthocyanin dilution and Biochemical laboratory as an apoptosis index and Fas expression in endometrial epithelial cells. Whereas purple sweet potato anthocyanin was obtained from the extraction, isolation, purification and characterization of anthocyanins carried out by Dr. Ciptati M.Sc., M.Sc at the Laboratory of FMIPA ITB. Based on the results of the Wiyasa (2012) study four weeks (28 days) after ovariectomy of white rats under hypoestrogenic conditions. The purple variety of sweet potato anthocyanin was given 4 weeks (28 days) after ovariectomy and was given for 30 days orally through a sonde. The research process began by acclimatizing 30 white mice that had met the inclusion and exclusion criteria for 7 days. Then white rats were grouped into 5 treatment groups. Performed maintenance for 2 months. After that termination is done by surgery and organ harvesting. The ovariectomy procedure was carried out after the acclimatization process that is on the 8th day using ketamine anesthesia 40-80 mg / kgBB (intraperitoneal) and xylazine 5-10 mg / kg BW (intraperitoneal). Then sampling is done to examine the Apoptosis Index using TUNEL Assay, examination of Fas expression using Immunohistochemistry (IHC), and staining of Hematoxylin Eosin (HE) in endometrial tissue.

IV. RESULT AND DISCUSSION

Fas expression was observed using the immunohistochemical method (IHC). Fas expression is clearly seen in the nucleus of endometrial epithelial cells using the Olympus microscope with 1000x magnification.

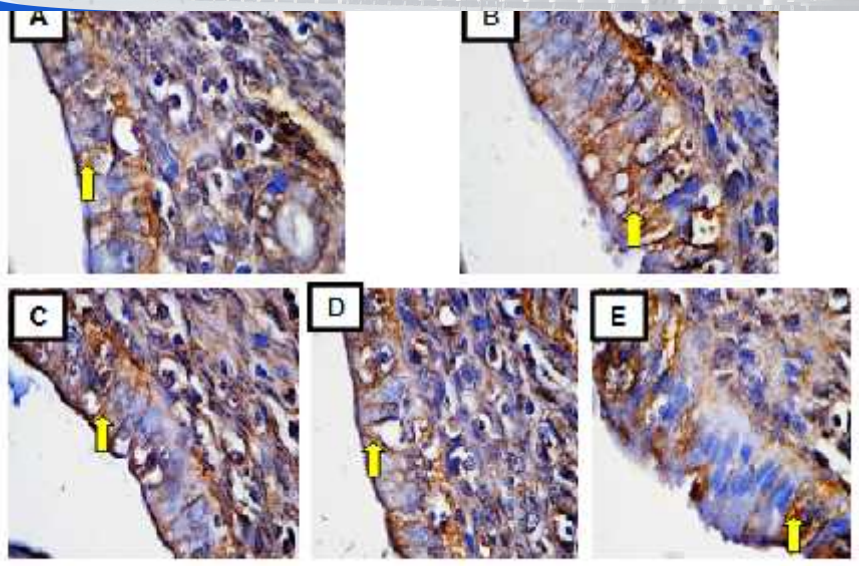

Figure 2. Ekspresi Fas pada Sel Epitel Endometrium

Calculation of Fas expression is done by counting the number of endometrial epithelial cells. Brown color divided by the total number of total cells observed each field of view. The number of Fas expressions is calculated in 20 visual fields with 1000x magnification. Marking the number of cells that have been calculated is marked using the help of raster image software. One Way Annova test was conducted and based on the test it was found that there were significant differences in the mean expression of Fas endometrial epithelial cells of the five treatment groups, this was indicated by the p-value = 0,000 <α. After that, a post-hoc LSD (Least Significant Difference) test was conducted to find out which groups had significant differences. Based on the results of LSD test, the results showed that there were significant differences in the expression of Fas between the positive control group (ovariectomy without anthocyanin) and the anthocyanin group 1 (ovariectomy + anthocyanin dose 20 mg / kg BW), anthocyanin group 2 (ovariectomy + anthocyanin dose 40 mg / kg BB) and anthocyanin group 3 (ovariectomy + anthocyanin dose 80 mg / kg BB). But there was no significant difference between the negative control group and the anthocyanin group 3 (Ovariectomy + anthocyanin dose 80 mg / kg BB). From these results it can be concluded that there is an influence of purple variety sweet potato anthocyanin on the expression of Fas ovariectomy endometrial epithelial cells. The optimal dose of purple sweet potato anthocyanin in this study was 80 mg / kg because the dose was able to reduce the expression of Fas to near normal conditions so that no dose was added because it might cause toxicity. Like previous studies which stated that administration of anthocyanin at a dose of 80 mg / kg BB in Galur Wistar white rats had histopathological changes in the liver. This shows that anthocyanin metabolism at high doses can turn into prooxidants (10).
The results of the One Way Annova and LSD tests can be seen in the table below:

Tabel 1. Influence of purple sweet potato *toward Fas Epitel of Ovariectomy of White Rat*

Treatment Group	n	Fas Expression	
		Average ± Stand.dev	*p-value*
Negatif Control (KN)	6	9,33 ± 3,27[a]	
Positive Control (KP)	6	30,67 ± 4,50[b]	
Antosianin 1 dose 20 mg/kg BB (KA1)	6	24,33 ± 3,45[c]	0,000 < α
Antosianin 2 doses 40 mg/kg BB (KA2)	6	15,67 ± 3,20[d]	
Antosianin 3 doses 80 mg/kg BB (KA3)	6	9 ± 3,29[a]	

V. CONCLUSION

The administration of purple sweet potato anthocyanin can reduce the expression of Fas ovariectomy in endometrial epithelial cells of white rat (Rattus norvegicus). The results of this study indicate that anthocyanin as an antioxidant capable of reducing Fas expression in white rat endometrial epithelial cells can be developed for further research with more varied anthocyanin doses.

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