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Original Article

Effect of Purple Variety Sweet Potato (*Ipomoea batatas* L.) Anthocyanin on Expression of Estrogen Receptor-A and Endometrium Thickness on Uterus of Female White Rats (*Rattus norvegicus*) Exposed to Cigarette Smoke

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Abstract

Cigarette smoke contains around 4000 chemical substances including free radicals and carcinogens, which are xenobiotic agents in the body, affecting the reproductive system. Purple sweet potatoes contain the color pigment anthocyanin. The purple color pigment (anthocyanin) in purple sweet potatoes is a beneficial antioxidant because it can react with free radicals that can cause damage in the body. The goal of the research was to understand the effects of purple variety sweet potato (*Ipomoea Batatas L*) anthocyanin on the expression of estrogen receptor-α in the endometrium and endometrium thickness on white rats (*Rattus norvegicus*) exposed to cigarette smoke. The White rats used for the 5 groups were 30 white rats. Exposure to cigarette smoke was given at a rate of 2 sticks/day (each cigarette ± 4 minutes), for 8 weeks after the rats were found to be in the proestrus phase. Endometrium thickness was examined and measured using a Dot slide Olympus XC 10 light microscope and the Olyvia software. Expression of ER-α in the endometrium was measured by using the antibody ER-α (C-311) SC-787 and the immunohistochemistry and calculated by the Immunoratio software. Data were analyzed with the Shapiro-Wilk's test, t-test and one-way ANOVA (F test). It was found that the average value of ER-α expression in the endometrium for the treatment group given purple variety sweet potato anthocyanin of a dose of 40 mg/Kg BW (15.51 ± 2.65) could increase ER-α expression in the endometrium of female white the rats exposed to cigarette smoke. The average value of endometrium thickness for the treatment group given purple variety sweet potato anthocyanin at a dose of 40 mg/Kg BW (152.51±9.59 µm) showed an increase in the endometrium thickness of female white rats exposed to cigarette smoke. The dose of purple variety sweet potato anthocyanin that could increase ER-α expression in the endometrium and endometrium thickness of rats exposed to cigarette smoke was a dose of 40 mg/Kg BW. The higher the dose of purple variety sweet potato anthocyanin, the greater the effect on the prevention of the reduction of estrogen receptor-α expression and the prevention of the reduction of endometrium thickness on female white rats exposed to cigarette smoke.

Keywords: cigarette, smoke estrogen receptor-α, endometrium thickness, *Ipomoea batatas L,* anthocyanin smoke

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1. Introduction

Smoking is one of the health problems present among people, causing 1 in 10 adult deaths and 5.4 million deaths across the world in 2006. By 2020, smoking-related deaths are estimated to increase two-fold if the habit of smoking is still prevalent among people [1].

In 2008, the World Health Organization (WHO) nominated Indonesia as the third-largest country of cigarette consumers after China and India [2]. According to the WHO, in 2004, second-hand passive smokers numbered 40% for children, 33% for non-smoking men, and 35% for women. According to GATS (Global Adult Tobacco Survey) in Indonesia (2011), non-smoking women (15 years or older) were exposed to cigarette smoke at work (41.2%), at home (75%), in government buildings (55.7%), at health facilities (16.5%), in places of eating (76%), in public transportation (62%), at universities (49%), at schools and educational facilities (31.9%), and in places of worship (12.7%).

Cigarette smoke contains around 4000 chemical substances including free radicals and carcinogens, which are xenobiotic agents in the body, affecting the reproductive system [3]. Free radicals in cigarette smoke, whether inhaled directly or indirectly, can cause oxidative stress [4].

The components of cigarette smoke that cause oxidative stress can then cause DNA damage to follicles in the ovaries, which are the source of the hormone estrogen [5]. Estrogen can increase the concentrating effect of estrogen receptor-α in reproductive organs, one of them being the endometrium [6].

An important component that is able to save human body cells from the damage of free radicals is antioxidants [7]. Antioxidants are substances that can inhibit or prevent the oxidation of other substances by free radicals [8]. Based on the source, antioxidants are grouped into three kinds: antioxidants in the body, synthetic antioxidants, and natural antioxidants [9]. Natural antioxidants originate from fruits, vegetables, grains, and tubers. One example of a source of natural antioxidants is the purple sweet potato.

Purple sweet potato (*Ipomoea batatas, L.*) has skin and tuber flesh which is colored dark purple. Purple sweet potatoes contain the color pigment anthocyanin. The purple color pigment (anthocyanin) in purple sweet potatoes is a beneficial antioxidant because it can react with free radicals that can cause damage in the body [10].

Research on anthocyanin and oxidative stress has often been performed, but research on the effects of anthocyanin on reproductive organs is still limited. The research done by Setyaningsih [11] covered soybeans, which contain isoflavones; its structures resemble the hormone estrogen, which can interact with estrogen receptors \reduce cholesterol content

through the same mechanism that regulates liposis, lipogenesis, and adipogenesis. In addition, the research conducted by Wahyuni *et al.* [12] showed that the combination of vitamin C and E was able to inhibit the toxicity of the endometrium caused by the treatment of MSG through the increase of angiogenesis and endometrium thickness, and the modulation of estrogen receptor-α.

Based on the explanation above, the researcher was interested in conducting research on the effect of purple variety sweet potato (*Ipomoea Batatas L*) anthocyanin on the expression of estrogen receptor-α and endometrium thickness on white rats (*Rattus norvegicus*) exposed to cigarette smoke.

2. Material and Methods

2.1. Research Design

The research design used in this research is a true experimental research with an approach using the posttest only control group design performed in the laboratory in an *in vivo* manner.

2.2. Research Subject

In this research, the samples used were white rats (*Rattus norvegicus*); in total, 30 white rats were used in 5 groups.

2.3. Research Variables

The research variables that were observed or measured covered the independent variable, the purple variety sweet potato anthocyanin of various doses, while the dependent variables were ER-α expression and endometrium thickness.

2.4. Procedure for Cigarette Smoke Exposure

Cigarette smoke exposure on the test animals utilized the smoking pump device created by the Pharmacology Laboratory of the Faculty of Medicine, University of Brawijaya. Cigarette smoke exposure was performed with 2 sticks/day (each cigarette ± 4 minutes), using one in the morning (1 stick) and one in the afternoon (1 stick) for 8 weeks after the rats were found to be in the proestrus phase. The location of cigarette smoke exposure (a fiberglass box of the size 26 cm x 12 cm x 12 cm) was only filled by three mice because the *smoking pump* only provided three spaces. The brand of cigarettes used was Gudang Garam Merah *kretek* cigarettes. For the subsequent exposure, the box was always cleaned of the cigarette smoke remains of the previous treatment.

The basis for determining the amount of cigarettes used in this research was the doses used in the previous research that proved that the exposure to cigarette smoke of 2 sticks/day given in the morning (1 stick) and afternoon (1 stick) for 8 weeks could increase the MDA content, reduce the number of primary, secondary and de Graaf follicles, and reduce estradiol content in the ovaries of female white rats [13].

2.5. Treatment of Purple Variety Sweet Potato Anthocyanin

The doses of purple variety sweet potato anthocyanin used in this research were based on prior research, which showedthat a dose of 80 mg/kg BW resulted in significant effects on increased testis weight, sperm movement,

and spermatogenesis cell density; in addition, such a dose of the anthocyanin extract could also reduce the concentration of 8-OHdG, which is the marker for oxidative stress in male rats after induction with *varicocele* [13]. Anthocyanin was given by measuring the predetermined dose, which was then diluted using 2.5 ml of water. The anthocyanin solution formed was delivered orally with a 1 ml pipette. Anthocyanin was given along with cigarette smoke exposure when the rats entered the proestrus phase after acclimatization.

2.6. Pap Smear Examination

This Pap smear was meant to determine the proestrus phase of the rats. The Pap smear was done at the beginning of the research as a marker for the rats and was also done on the final day of the research with the goal to determine the estrous phase.

2.7. Procedure for Sample Extraction

Surgery was done after 8 weeks of treatment.

2.8 Measurement of Endometrium Thickness

Measurement of the endometrium thickness was done using a Dot slide light microscope Olympus XC10 and the Olyvia software.

2.9. Measurement of ER-A Expression in the Endometrium

Measurement of ER-α expression was done by using the antibodies ER-α (C-311) SC-787, LOT#J0914, *Mouse monoclonal* IgG_{2a} Santa Cruz Biotechnology.

3. Results and Discussion

3.1. Subject Characteristics

Measurement of ER-α expression in the endometrium used the *Immunohistochemistry*

Table 1. Results of Data Normality Test.

Observation groups	*p-value*		distribution
	ER-α expression	**endometrium thickness (µm)**	
Negative control	0.094	0.061	normal
Positive control (cig. smk.)	0.233	0.542	normal
A1 (cig. smk. + anto 20 mg/kg BW/day)	0.970	0.885	normal
A2 (cig. smk. + anto 40 mg/kg BW/day)	0.067	0.127	normal
A3 (cig. smk. + anto 80 mg/kg BW/day)	0.424	0.589	normal

Note: If p-value < 0.05, data did not have normal distribution, and if p-value >= 0.05, data had normal distribution.

Table 2. Results of control groups comparisons.

Variables	Negative control (healthy) Average ± std. dev.	Positive control (cig. smk.) Average ± std. dev.	*p-value*
ER-α expression	21.81 ± 2.84	11.09 ± 0.73	0.000< α
Endometrium thickness	216.75 ± 46.99	85.11 ± 13.82	0.000< α

(IHC) method and endometrium thickness was seen with *Hematoxylin Eosin* (HE) staining. (Figure 1 and 2)

3.2. Results of Prerequisite Parametric Testing

In table 1, based on the *Shapiro-Wilk* test, it was found that data for ER-α expression in the endometrium (%) and endometrium thickness (μm) for each of the observation groups showed p-values which were all greater than the significance level of α = 0.05. Thus, all of the data fulfilled the prerequisite parametric test, having been proven to have normal distribution.

3.3. Results of Comparison between Control Groups

The results of comparison testing between the negative control (healthy rats) and the positive control (rats exposed to cigarette smoke) groups for ER-α expression in the endometrium (%) and endometrium thickness (μm) using the independent sample t-test is explained and concisely presented in the table 2.

In table 2, based on the independent sample t-test, it was found that there was a significant difference ($p = 0.000 < \alpha$) in the average expression of ER-α between the negative control (healthy rats) (21.81 ± 2.84%) and positive control (rats exposed to cigarette smoke and not given anthocyanin) (11.09 ± 0.73%) groups. There was also a significant difference ($p = 0.000 < \alpha$) in the average endometrium thickness between the negative control (healthy rats) (216.75 ± 46.99 μm) and the positive control (rats exposed to cigarette smoke) (85.11±13.82 μm) groups.

Based on the above explanation, it can be said that rats exposed to cigarette smoke show a reduction of ER-α expression in the endometrium and endometrium thickness.

3.4. Results of Comparison of ER-A Expression in the Endometrium

Based on the results of one-way ANOVA on the data for ER-α expression, it was found that there was a significant difference in the average ER-α expression of the five sample observation groups; the multiple comparison testing with the Least Significant

Table 3. Comparison of the effects of purple sweet potato anthocyanin on ER-α expression (%).

Observation groups	n	Average ± std. dev.	p-value
Negative control	6	21.81 ± 2.84^{a}	$0.000 < \alpha$
Positive control (cig. smk.)	6	11.09 ± 0.73^{b}	
A1 (cig. smk. + anto 20 mg/kg BW/day)	6	13.05 ± 1.05^{bc}	
A2 (cig. smk. + anto 40 mg/kg BW/day)	6	15.51 ± 2.65^{cd}	
A3 (cig. smk. + anto 80 mg/kg BW/day)	6	16.98 ± 2.27^{d}	

Note: Results of the LSD test are shown in the average ± std. dev. column; different letters represent significant differences (p-value < 0.05) and same letters represent no significant differences (p-value > 0.05).

Difference/LSD testing are presented in full in table 3.

Based on table 3, it can be reasoned that the treatment of purple sweet potato anthocyanin at a dose of 40 mg/Kg BW had a significant effect on the increase of ER-α expression on rats exposed to cigarette smoke. Thus, the first research hypothesis was proven in that the purple variety sweet potato anthocyanin could increase ER-α expression on rats exposed to cigarette smoke. In addition, the dose of sweet purple variety

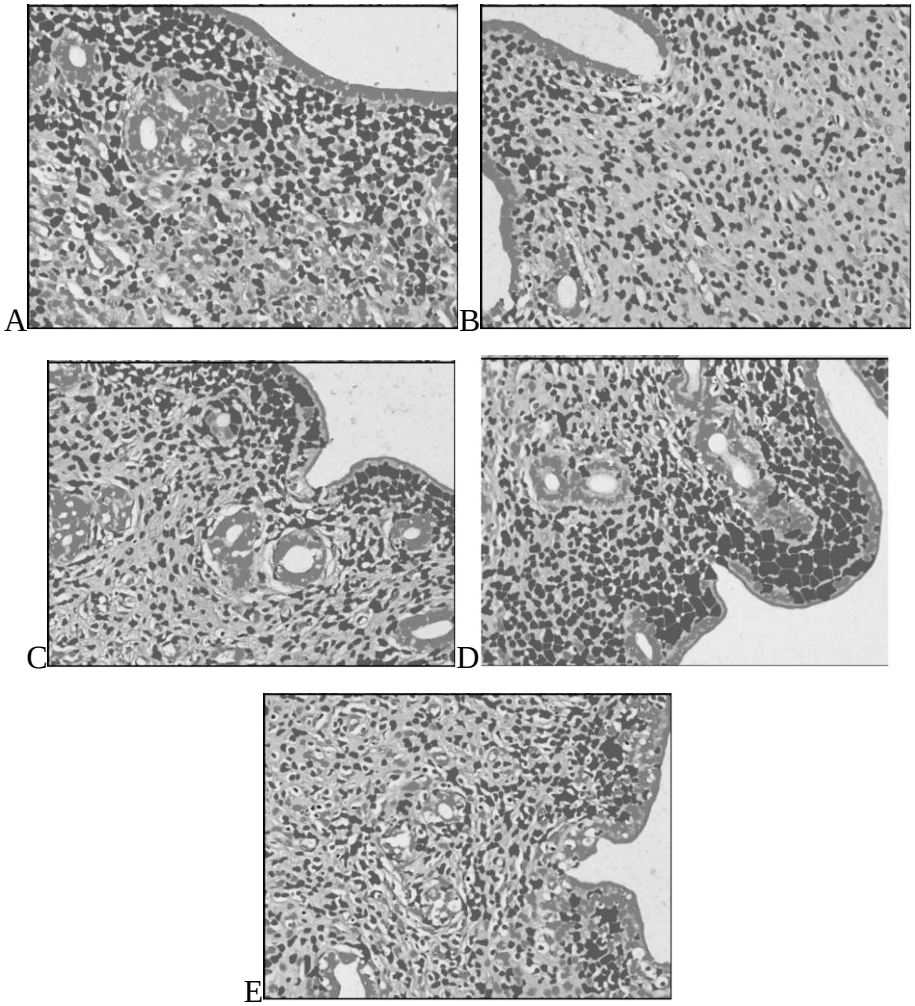

Figure 1. Observation Results of ER-α Expression in the Cell Nucleus. (A) Negative Control. (B) Positive Control. (C) Anthocyanin of 20 mg/Kg BW Dose. (D) Anthocyanin of 40 mg/Kg BW Dose. (E) Anthocyanin of 80 mg/Kg BW Dose.

Notes: Measurement of estrogen receptor-α (ER-α) expression in the endometrium was done by the IHC (Immunohistochemistry) method. Results of the preparations stained for IHC was then photographed with an OLYMPUS DP71 microscope with 400x magnification of 10 fields. The photos were then put into the Immunoratio software. It was found that there were differences of ER-α expression in the cell nuclei represented by the brown color (shown by the arrow) of the negative control, positive control, anthocyanin of 20 mg/Kg BW dose, anthocyanin of 40 mg/Kg BW dose, and anthocyanin of 20 mg/Kg BW dose groups.

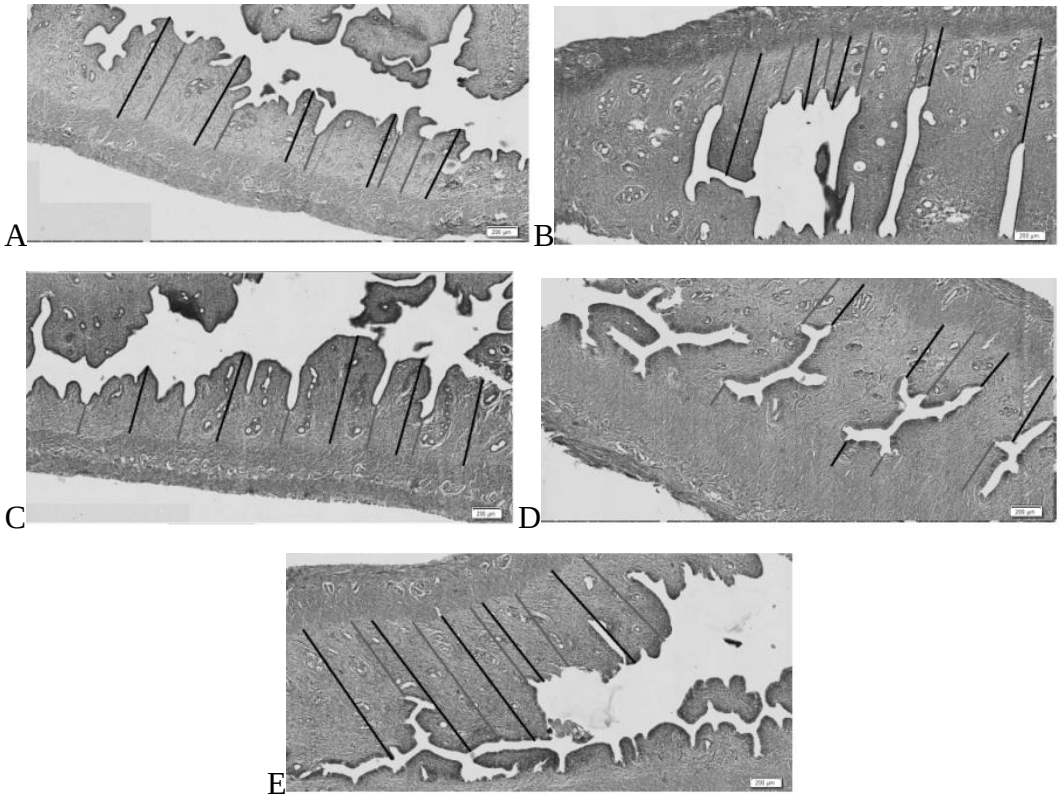

Figure 2. Microscopic Observation Results of Endometrium Thickness. (A) Negative Control. (B) Positive Control. (C) Anthocyanin of 20 mg/Kg BW Dose. (D) Anthocyanin of 40 mg/Kg BW Dose. (E) Anthocyanin of 80 mg/Kg BW Dose.

Notes: Measurement of endometrium thickness was done using the HE (*Hematoxilin Eosin*) method. Results of the preparations which were stained with HE were then scanned with an OLYMPUS XC10 microscope. The scan results were then put into the OLYVIA software to measure the average endometrium thickness with 400x magnification of 10 points (5 highest thick points are shown by black lines; 5 lowest thick points are shown by red lines). From the measurement results it was found that there was a difference in endometrium thicknesses of the negative control, positive control, anthocyanin of 20 mg/Kg BW dose, anthocyanin of 40 mg/Kg BW dose, and anthocyanin of 20 mg/Kg BW dose groups.

sweet potato anthocyanin that was considered most significant in increasing the expression of ER-α in the endometrium was a dose of 40 mg/Kg BW.

The average ER-α expression of the five sample groups is presented in full in the histogram below.

In figure 3, it can be seen that there was an increase in the average ER-α expression along with increased treatment doses of the purple variety sweet potato anthocyanin. The average value of ER-α expression in the endometrium was highest in the groups with purple variety sweet potato anthocyanin treatment at a dose of 40 mg/Kg BW (15.51 ± 2.65). As such, in this research, the dose of purple variety sweet potato anthocyanin that is considered to significantly increase ER-α expression in the endometrium on rats exposed to cigarette smoke is a dose of 40 mg/Kg BW.

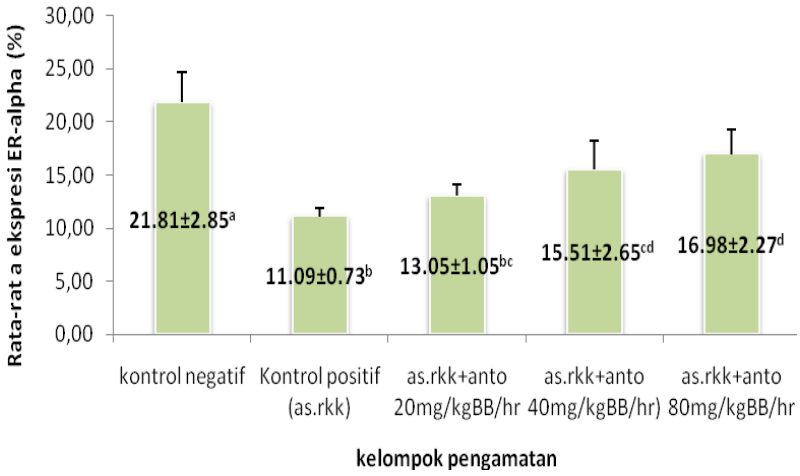

Figure 3. Histogram of average ER-α expression.

Based on the results of the independent sample t-test between the negative control group (healthy rats) and positive control group (rats exposed to *kretek* cigarette smoke from 2 sticks per day and not given anthocyanin treatment), it was shown that the average expression of ER-α showed a larger value in the negative control group (96.66%) compared to the average expression of ER-α for the positive control group. This means that rats exposed to cigarette smoke show lower ER-α expression compared to the healthy rats.

This is in line with the research by Febriyani [14], showing that cigarette smoke can reduce the estradiol hormone content of female white rats. It was explained that cigarette smoke contains many toxic substances such as free radicals, nicotine, carbon monoxide, and tar, which affect nerve cells in the brain. These nerve cells would affect the secretion of estradiol. According to Dechanet *et al.* [15], substances from cigarette smoke act to disturb steroidogenesis which leads to the reduction of estradiol synthesis and a deficiency of progesterone. Research performed by Bersthein *et al.* [16] stated that exposing rats to cigarette smoke for 3 months resulted in specific reductions in the estrogen hormone and the induction of progesterone receptors in uterine tissue.

Also, according to Dechanet *et al.* [15], cigarette smoke is related to disturbances in folliculogenesis. Ovaries containing substances from cigarette smoke result in follicles being exposed to toxic substances, inducing the increase of oxidative stress, *cross-talk* between abnormal cells, and a reduction of meiosis, as well as the activation of cell apoptosis paths in the ovaries. The consequences are the loss of follicles, abnormal follicle growth, morphologic disturbances of oocytes, and maturity of oocytes.

The reduced stimulation of estrogen causes a reduction in the amount of estrogen receptors and reduced affinity to the endometrium [17]. Loose and Stancel [18] reported that ER-α was most expressed in the female reproductive tract, especially in the uterus, vagina, ovaries, breast glands, hypothalamus, endothelium cells, and vascular cells of the smooth muscle. The function of ER-α is more distributed in the structural tissues of reproductive organs; as an example, ER-α is dominant in the uterus, stromal cells, theca cells, and bone. ER-α is far greater than ER-β (by about 100:1) in a normal endometrium [19, 20]. The uterus is the reproductive organ that possesses estrogen receptors, so that changes that occur in the structural layers of the uterine wall are the result of hormone regulation, especially of the estradiol hormone [21].

The absence of the hormone estrogen in the endometrium will cause estrogen receptors to become inactive. The optimal amount of estrogen will bind to the estrogen receptors in the core and cause estrogen receptors to become active [22]. Estrogen must penetrate the cell surface, enter the cell (cytoplasm), and then bind to estrogen receptors. The cytoplasm forms receptor hormone bonds with the *Estrogen Response Element* (ERE) and then moves into the cell nucleus to bind with DNA. Estrogen receptors that have bound DNA play a role in the cell transcription process to form proteins that are necessary for cell division [23].

Based on the ANOVA test, along with the LSD test, there was no significant difference in the average expression of ER-α between the positive control group and the treatment group exposed to cigarette smoke and given purple variety sweet potato anthocyanin of a dose of 20mg (17.67%). Although there was an increase in ER-α expression for the 20 mg dose group, this increase was not statistically significant. Meanwhile, the groups given doses of 40 mg and 80 mg showed a significant difference in the expression of ER-α by 39.85% and 53.11%. This is in accordance with the research conducted by Scorvita [24], where the treatment of ethanol extract of mangosteen rind was able to inhibit the reduction of total testosterone content in the blood of male Wistar rats exposed to cigarette smoke due to the various substances contained in mangosteen rind, among them mangostin, tannin, xanthone, crysanthemin, garcinone, gartanin, vitamin B1, B2, terpene, anthocyanin, phenol, and other bioactive substances.

Anthocyanin is a type of polyphenol belonging to flavonoids, which can directly or indirectly reduce oxidative damage by preventing the increase of free radicals in the body. According to Kumalaningsih [10], anthocyanin is a flavonoid and a polyphenol substance that functions as an antioxidant. Flavonoids belong to the group of phytoestrogens, which are estrogen-like substances of plant origin that are non-steroidal and have estrogenic effects. According to Satyaningtijas [25], the ethanol extract of *purwoceng* (*Pimpinella pruatjan*), which contains flavonoids, as substances that are estrogenic in nature, can function as

estrogen in the body, which will increase estrogenic effects. Flavonoids that are estrogenic in nature can sit in estrogen receptors of the body and create estrogen-like effects.

3.5. Results of Comparison for Endometrium Thickness

Based on the results in table 4, it can be reasoned that the treatment of purple sweet potato anthocyanin at a dose of 40 mg/Kg BW had a significant effect on the increase of endometrium thickness on rats exposed to cigarette smoke. Thus, the second research hypothesis demonstrated that the treatment of purple variety sweet potato anthocyanin could increase endometrium thickness on rats exposed to cigarette smoke. In addition, the dose of sweet purple variety sweet potato anthocyanin that was considered most significant in increasing the endometrium thickness was a dose of 40 mg/Kg BW.

The average endometrium thicknesses of the five sample groups are presented in full in the histogram below:

Figure 4 shows the histogram of average endometrium thicknesses of the negative control group (healthy rats), positive control group (rats exposed to cigarette smoke), and 3 groups of rats exposed to cigarette smoke and given treatments of purple variety sweet potato anthocyanin using doses of 20 mg, 40 mg, and 80 mg. It can be seen that there was an increase in the average endometrium thickness with a treatment dose of 40 mg/Kg BB.

The highest average value of endometrium thickness was in the purple variety sweet potato anthocyanin treatment group with a dose of 40 mg/Kg BB (152.51 ± 9.59 µm). As such, in this research the dose of purple variety sweet potato anthocyanin that is considered to significantly increase endometrium thickness in rats exposed to cigarette smoke is a dose of 40 mg/Kg BW.

Table 4. Comparison of the effects of purple sweet potato anthocyanin on the endometrium thickness (µm)

Observation groups	n	Average ± std. dev.	p-value
Negative control	6	216.74 ± 46.98^{d}	$0.000 < \alpha$
Positive control (cig. smk.)	6	85.11 ± 13.82^{b}	
A1 (cig. smk. + anto 20 mg/kg BW/day)	6	101.53 ± 6.89^{b}	
A2 (cig. smk. + anto 40 mg/kg BW/day)	6	152.51 ± 9.60^{c}	
A3 (cig. smk. + anto 80 mg/kg BW/day)	6	165.03 ± 12.96^{c}	

Note: Results of the LSD test are shown in the average ± std. dev. column; different letters represent significant differences (p-value < 0.05) and same letters represent no significant differences (p-value > 0.05).

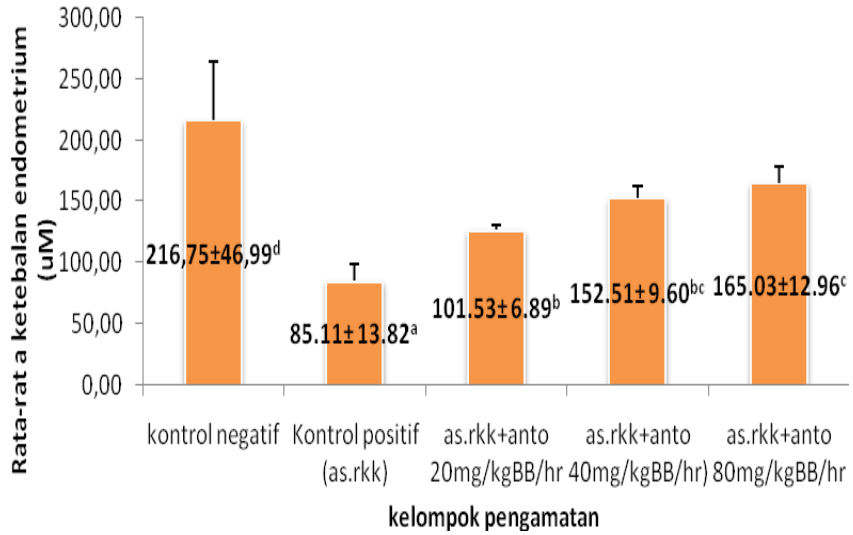

Figure 4. Histogram of average Endometrium Thickness.

Based on the result of calculations of the independent sample t-test between the negative control group and the positive control group (exposed to cigarette smoke from 2 sticks per day and not given anthocyanin treatment), the average endometrium thickness in the negative control group was larger in value (154.67%) when compared with the average endometrium thickness of the positive control group. This means that the rats exposed to *kretek* cigarette smoke from 2 sticks per day and not given treatment of anthocyanin showed a very low endometrium thickness when compared with healthy rats. The results of the multiple comparison test with the LSD test showed that there was a significant difference in the average endometrium thickness between the positive control group and the treatment group given purple variety sweet potato anthocyanin of doses 40 mg (79.19%) and 80 mg (93.90%), but insignificant for a dose of 20 mg (19.29%). This means that there was an effect of the treatment of purple variety sweet potato anthocyanin of 40 mg and 80 mg doses on the endometrium thickness of rats exposed to cigarette smoke. The effect of the purple variety sweet potato anthocyanin was to increase the endometrium thickness. An increase in the endometrium thickness occurred along with increased doses of purple variety sweet potato anthocyanin given to rats exposed to cigarette smoke.

This is in line with that which was reported by Dechanet *et al.* [27], who showed that substances in cigarette smoke such as benzo (a)pyrene, nicotine, and cadmium disrupt the cytochromes involved in the metabolism of estrogen so that they may act as anti-estrogenic factors that would reduce the maturity of the endometrium, including the reduction of thickness of the epithelial layer of the endometrium and inflammation of the stromal cells of the endometrium.

The concentration of estradiol in the endometrium is significantly higher compared to the periphery circulation [26]. Estradiol is produced in the ovaries through the aromatase

steroid process by granulose cells, so that as more follicles are formed, the production of estradiol also increases. During the pre-ovulation or follicular phase, the concentration of circulating FSH is low, but because the concentration of estrogen and inhibin increase, both continuously give feedback to suppress the release of FSH. Negative feedback from estrogen keeps LH low during the early follicular phase. The increase in estrogen concentration keeps LH low at the early follicular phase. The increase in estrogen concentration at the end of the follicular phase sensitizes gonadotropin hypophysis toward GnRH so that the rise in LH is still pre-ovulation and triggers ovulation and proliferation in epithelial and stromal cells in the endometrium [28, 29].

The endometrium is an organ that is very responsive toward changes in reproductive hormones, such as the hormone estrogen, which plays a role in proliferation [30]. Changes in the endometrium thickness are regulation for changes of the estrogen hormone. The decreasing thickness of the endometrium wall will cause disturbances in zygote implantation which will reduce the possibility of pregnancy. This is supported by the research of Putri [30] that the thinner the endometrium becomes, the smaller the rate of pregnancy, which is related to the function of the ovaries and endometrium.

During the reproductive period, the endometrium is dynamic and will undergo a cycle of proliferation, differentiation, and shedding. The endometrium is expected to be proliferative or secretory, depending on the phase of the menstrual cycle in pre-menopausal women [32]. The ability of the endometrium to provide an environment that is perfect for conception, implantation, the start of gestation, and placentation is an important point for pregnancy and fertility [33].

The effect of cigarette smoke can also be seen in the endometrium blood flow, sub-endometrial vascularization, and intensity of blood flow during the normal menstruation cycle. BAP (Benzo (a)pyrene) can affect the ovaries so that the production of estrogen will be disrupted and affect the blood flow of the endometrium during the menstruation cycle, thus reducing the thickness of the endometrium [34, 35]. Research conducted by Khorram, *et al.* [36], using a culture of human endometrium epithelial cells, reported that cigarette smoke could reduce the proliferation of the endometrium through the nitrogen oxide path. Cigarette smoke has been linked to infertility, abnormal uterine bleeding, an increased risk of endometrial cancer to pre-menopausal women, and stimulation of the production of nitrogen oxide in cells of the endometrium.

4. Conclusion

In this research, it can be concluded that the higher the dose of anthocyanin of purple variety sweet potatoes (*Ipomoea batatas, L.*), the larger the effect towards the prevention of reduction of the expression of estrogen receptor α and the prevention of reduction of the endometrium thickness of white rats exposed to cigarette smoke.

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