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The Effect Of Administering Extract Of Java Plum Ethanol (*Syzygium Cumini*) to The Expression Of The Molecular Adhesion 1 (Icam-1) On The Model Of Huvecs Preeclampsia

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ABSTRACT

Introduction: Preeclampsia is one of the causes of maternal mortality. In preeclampsia, endothelial dysfunction triggers the emergence of inflammatory cytokines and increases the expression of the molecular adhesion of ICAM-1. Inflammation of the preeclampsia can be controlled with the administration of anthocyanins. Java plum (Syzygium Cumini) contains anthocyanins that serve as antioxidants and anti-inflammatory.
Purpose: This research aims to prove the influence of the administration of java plum (Syzygium Cumini) extracts on the expression of ICAM-1 on the model of HUVECs preeclampsia.
Method: The research is conducted experimentally. It is done within the glass in a laboratory with two control groups namely negative control, three positive control, and six experimental groups (100ppm dose, 200ppm and 400ppm, incubation 1 hour, 3 hours, and 24 hours). HUVECs preeclampsia as a model of HUVECs preeclampsia within the glass. The ICAM-1 expression is used in the immunohistochemistry. The statistical analysis uses Two Way Anova test and regression test.
Result: at the time of incubation of 1 hour, 3 hours, and 24 hours, there is no meaningful difference in the expression ICAM-1 ($p = 0.392 > \propto$). At the dose ICAM-1 of java plum, there is a meaningful difference between the positive control group with the dosage group of ($p = 0.000 < \propto$).
Conclusion: An expression of ICAM-1 increases in preeclampsia conditions, administering java plum at a dose of 100 ppm, 200 ppm, and 400 ppm may decrease the expression of ICAM-1 in preeclampsia condition.

Keywords: Java Plum, Preeclampsia, ICAM-1

BACKGROUND

Preeclampsia is a specific pregnancy disorder characterized by hypertension and proteinurine occurring after the 20th week of pregnancy. The trophoblasts invasion on the uterine spiralist artery with the clinical symptoms of hypertension, proteinuria and edema, derived from pathological changes in the vascularity of the endothelium mother or resulting in endothelial dysfunction of the

placenta (Keiichi, et al., 2015). Vasochtrixy perfusion of Uteroplasenta induced trophoblastic cells that failed to conduct remodeling and adhesion to spiralist artery walls. This trigger increased the expression of the ICAM-1 adhesion molecule to the surface of the endothelial cell that interacts with its ligand on the surface of leukocytes (Elizabeth, et al., 2016). In vitro research models can be performed using preeclampsia plasma which induces inflammatory cytokines.

The emergence of inflammatory cytokines results in increased activity of macrophages and netrophiles in the blood vessels of the spiralist arteries, resulting in endothelial dysfunction. Endothelial dysfunction is the failure of endothelial in conducting an adequate adaptation to stimulation caused by exposure to inflammatory cytokines and increased expression of the molecular adhesion of ICAM-1 so that there is oxidative stress. Oxidative Stress on Preeclampsia can be controlled by the administration of antioxidants. Antioxidants are widely found in fruits. Java plum (Syzygium cumini) contains anthocyanins, phenols and polyphenols, a compound that serves as an antioxidant and anti-inflammatory (Ananda & Nurul, 2016). Java plum's ethanol extract has a higher concentration of anthocyanins compared to isopropanol extract (Ananda & Nurul, 2016). Use of ethanol because the compounds in the water-soluble of Java plum. In addition, ethanol has the properties of dissolving almost all substances either polar, nonpolar and semipolar. Java plum's extract starts the dosage of 100, 200 and 400 ppm on the preeclampsia HUVECs model significantly lowers TNFά levels. Java plum's extracts can decrease the suspected oxidized lipid through the decline of TNFά (Baktiyani, 2009) will affect the expression of ICAM-1 (Baratawidjaja & Rengganis, 2010). In this research, there will be research on the influence of the introduction of Java plum's extracts (Syzygium cumini) on the Model of the HUVECs plasma preeclampsia against the expression of adhesion molecule 1 (ICAM-1) and molecular cell adhesion 1 (VCAM-1).

OBJECTIVE

Is there any influence of the extract of Java plum (Syzygium cumini) in the expression of adhesion molecule 1 (ICAM-1) on the Model HUVECs preeclampsia?

METHOD

This research is an experimental study done in vitro laboratory by using an experimental design in the form of a completely randomized design (CRD) with two control groups, namely negative control, three positive controls, and six experimental groups. Research conducted at Gamma Scientific Biolab Malang. The research time was December 2019-January, 2020. The research uses endothelial cell samples derived from the newborn umbilical cord-derived from maternity mothers with section Caesarea (SC) with Preeclampsia. Umbilicus was taken through Caesar's delivery with inclusion criteria as in the operational definitions. Endothelial cell culture works not exceeding 12 hours after birth time. Prepared bottles are containing cord solution from the refrigerator (temperature 4 º C). Extraction techniques using the maceration technique, the HUVECs culture manufacture, is carried out based on the method of Morgan (1996), which has been modified by Gamma scientific Biolab Malang. Immunohistochemistry staining of ICAM-1 for 2 days. They are processing the data by observing the brown colour of the cell within one slide. DAB is used to visualize the yellowish-brown colour of an immunoreactive endothelial cell to an anti-ICAM-1 antibody. The results of staining molecules are analyzed in discrete—data in the form of a per cent (%) of which is expressed. Data analysis is conducted two phases of consecutive counting, namely data normality test with Shapiro-Wilk test and Two-Way ANOVA test. Then, the hypothesis is chosen by using parametric statistic approach before testing with the parametric test; the data was previously tested with parametric requirement tests, i.e., samples of measured variables were tested first, whether data was dispersed or normal. This research has been through a test of ethics.

Results

Table 1 The Comparison among Java Plum Dosage

Observation Group	ICAM-1	
	Average ± SD	ρ
Positive Control (HUVECs Preeclampsia)	85.78±10.10[a]	0.000
HUVECs Preeclampsia+Java plum 100 ppm	67.58±12.61[ab]	

HUVECs Preeclampsia+Java plum 200 ppm	53.46±17.56^{bc}
HUVECs Preeclampsia+Java plum 400 ppm	41.15±16.70^{c}

Table 1. Based on multiple comparison test results with Tukey HSD test shows that there is no meaningful difference in the average of ICAM-1 among the positive control groups (85.78 ± 10.10%) With HUVECs Preeclampsia + Java Plum 100 ppm (67.58 ± 12.61). Based on its average value, it appears to indicate a decrease in the ICAM-1 of HUVECs Preeclamsi + 100 ppm, but the decline is not statistically meaningful. It means that the treatment of HUVECs Preeclampsia + Java Plum 100 ppm is considered no effect on the ICAM-1. However, the average ICAM-1 between the positive control group (85.78 ± 10.10%) With HUVECs Preeclampsia + Java Plum 200 ppm (53.46 ± 17.56%) or with HUVECs Preeklamsi + Java plum 400 ppm (41.15 ± 16.70%), indicating there is a meaningful difference. It means that there is an influence on the treatment of HUVECs Preeclampsia + Java Plum 200 ppm and HUVECs Preeclampsia + Java Plum 400 ppm treatment against the ICAM-1, namely being able to lower the ICAM-1. While the treatment of HUVECs Preeklamsi + Java plum 200 ppm (53.46 ± 17.56%) and treatment HUVECs Preeclampsia + Java Plum 400 ppm (41.15 ± 16.70%) There are no meaningful differences in the average of ICAM-1. It means that a dose of 200 ppm and 400 ppm has the same ability in terms of lowering the ICAM-1. However, based on the average value of ICAM-1 looks the lowest is in the treatment group HUVECs Preeclampsia + Java Plum 400 ppm. From the results of Anova two way above, it can be inferred that there is an effect of HUVECs Preeclampsia + Java plum on ICAM-1.

Table 2 The Result of Regression Analysis of Java Plum Influence on ICAM-1

Dependent Variable	Regression Coefficient	**r**	**r^2 x 100%**	***p***
ICAM-1	$\hat{y}$ = 80.494 – 0.132 x	-0.587	34.5%	0.001

Table 2. it explains that the regression results indicate there is a meaningful influence on the ICAM-1 with the equation linear model yˆ= 80.494-0.132 x or ICAM-1 = 80,494 – 0.132 (Java plum) (p = 0.001 < ∝). With a correlation coefficient of R =-0587 which means there is quite a meaningful relationship between the Java plum with the ICAM-1. Negative value-0587 is the opposite of a relationship between the Java plum t with the ICAM-1, that is, if the dose is increased, the ICAM-1 will decrease or close to the condition without the Java plum viewed. While in this research the percentage of the influence of Java plum the ICAM-1 is 34.5% and ICAM-1 is influenced by besides Java plum is at 65.5%. Connection of the Java plum dose against the ICAM-1 graph expressed in the form of the relationship shown below.

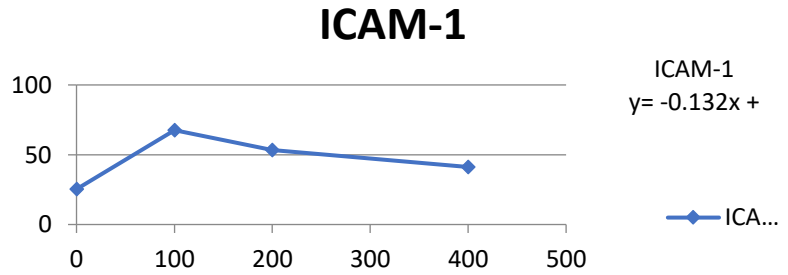

Figure 1. Regression Coefficient Model of ICAM-1

In Figure 1, it explains that there is a linear relationship of meaningful influence between the doses of Java plum against the ICAM-1 of regression analysis results with a regression model yˆ= 80.494-0.132 x.

DISCUSSION

An expression of ICAM-1 on HUVECs that has a plasma preeclampsia number continues to increase, indicating a correlation that the number of ICAM-1 expressions increased in endothelial cells activated by TNF-α, IL-1 or endotoxin (Baratawidjaja & Rengganis, 2010). Inflammatory cytokines can be found in serum and plasma preeclampsia. Plasma patients with Preeclampsia besides containing oxidative stress ingredients, contained cytokine material (TNF-α, Il-6), ANG II (Baktiyani,

2009). These cytokines are suspected of inducing inflammatory reactions of the HUVECs culture. So that if the culture of HUVECs preeclampsia containing oxidative stress and inflammatory material is suspected HUVECs produce higher anions superoxide (O2-) and will increase the expression of the molecular adhesion of ICAM-1 on the surface of endothelial cells that interact with its ligation on the surface of leukocytes (Baratawidjaja & Rengganis, 2010).

The plasma of patients with Preeclampsia besides containing oxidative stress ingredients, contained cytokine material (TNF-α, Il-6), ANG II (Elizabeth, et al., 2016). Such cytokines may induce inflammatory reactions to the culture of HUVECs preeclampsia. So that if the culture of HUVECs preeclampsia containing oxidative stress material and inflammatory material allegedly HUVECs produce anion superoxide (O2-) higher. The culture of HUVECs preeclampsia has occurred increased levels of H2O2 compared to H2O2 levels in the blood plasma of women with normal pregnancies, PEB and eclampsia. Suspected HUVECs culture produces higher anions superoxide (Baktiyani, et al., 2007).

Anion superoxide, hydrogen peroxide and the presence of transition metals initiate a chain of lipid peroxide reaction. Anion superoxide (O2-), mutated by the enzyme SOD (superoxide dismutase) produces hydrogen peroxide (H2O2) due to the presence of transition metals, such as Fe2 +, CU2 + will produce highly reactive hydroxyl radicals (O2-+ H2O2 OH + OH-+ O2) (Miguel, 2011). Hydroxyl radicals will attack cell membranes, double bonds of unsaturated fatty acids/polyunsaturated fatty acids (LH) so that it forms radical lipid (L.). This radical lipid interacts with the oxygen molecule (O2) into lipid peroxide. Lipid peroxide and increased oxidative stress and may result in decreased antioxidant enzymatic (Miguel, 2011).

Endothelial cell cultures can be activated via monocyte activation. Activation of monocytes releases free radicals, thereby increasing the activity of surface cells. The surface activity of cells will trigger an ICAM-1 expression. The granting of the ICAM-1 antibodies on the HUVECs will react with the ICAM-1 molecule that appears on the surface of the cell, indicating inflammation. During inflammation, the cell will produce cytokines that can express the adhesion molecule. This condition results in a higher inflammatory response. The inflammatory response activates endothelial cells and higher macrophage cells resulting in a systemic inflammatory reaction to Preeclampsia (Lawson & Wolf, 2009).

The results were in accordance with Serano's research (2006), stating that the increase in ICAM-1 occurred in Preeclampsia caused by Throfoblast, an invasion of decidua and remodelling in spiral arteries. According to Lawson & Wolf (2009) stating that in normal pregnancy, the inflammatory process occurs but still within normal limits. In this study, the culture of HUVECs that was displayed by plasma preeclampsia has occurred increased expression of ICAM-1 allegedly due to the absence of inflammatory cytokines and the occurrence of oxidative stress.

In this research, the expression of ICAM-1 in HUVECs preeclampsia was then administered Java Plum at a dose of 100ppm, 200ppm, and 400ppm, and each dose incubated 1 hour, 3 hours and 24 hours, decreased in the presence of reduced amount of brown color on the cytoplasm. Based on the results of a regression test with a correlation coefficient of R =-0587, which means there is quite a meaningful relationship between Java Plum with ICAM-1. The negative value is 0587 that there is an inverse relationship between Java Plum and ICAM-1; that is, if the dose of Java plum increases, then the ICAM-1 will decrease or vice versa. While in the study, the percentage of influence of Java plum to the ICAM-1 is 34.5% and ICAM-1 is influenced by other than Java Plum is 65.5% (genetic factor, the role of Prostacyclin and Thromboxsan). It means that the treatment of Java Plum Administration can lower the ICAM-1 on huvecs that is displayed by plasma preeclampsia.

According to the study Libby (2002), which states the decline of the expression ICAM-1 decreases with the administration of antioxidants and anti-inflammatory. Based on the research, the fruit containing antioxidant and anti-inflammatory found in anthocyanins on Java plum fruit. Chemically, anthocyanins are the result of polyhydroxy glycosylation and or polymethoxy derivatives of 2-benzopirilium salts or known as flavilium structures. It is supported by research conducted Lestario (2003) that the anthocyanins levels in the fruit of Java plum are getting higher then there is an increase in antioxidant activity and anthocyanins indicated by decreasing the oxidation levels of linoleic acid-producing peroxide.

In this study, It is reported that a decrease in the expression of ICAM-1 on HUVECs Preeclampsia allegedly due to the administration of Java plum extract containing antioxidant and anti-

inflammatory. Antioxidants play an essential role in preventing oxidative stress, and at the beginning, the formation of placenta began to emerge generation peroxide lipids on Preeclampsia through excessive production of superoxide anions that react quickly with NO to form peroxynitrite. According to Miguel (2011), the antioxidants that are given on the HUVECs can have different functions according to antioxidant activity. Antioxidant function in HUVECs, i.e. (a) decreased local oxygen concentration, (b) prevent the initiation chain with reactive oxygen and or nitrogen species (ROS/RNS), e.g., anion superoxide, hydrogen peroxide, radical hydroxyl, peroxynitrite, (c) binding to metal ions so that it will not produce species such as HO ⁻, ferryl or Fe2 +/Fe3 +/O2, and lipid peroxide. However, this study was not conducted a phytochemical test, so it is not known antioxidant activity in the Java plum extract.

In the study, it was reported that a decrease in the expression of ICAM-1 after the inclusion of the Java plum extract on the Model HUVECs Preeclampsia allegedly because it was influenced by decreased expression of NF-κB. It is also supported by the research conducted by (Baktiyani, 2009), stating that the Java plum extract can lower TNF-α and the NF-κB expression on the Model of the HUVECs that has the Preeclampsia Plasma. According to Inoue (2006), which states the decreased expression of TNF-α will biologically decrease the expression of ICAM-1, P-selectin, tissue factor, cytokines, and Chemokine. According to Lestari (2003), in Safitri (2011), decreased oxidation levels of linoleic acid-producing peroxide. Free radicals are oxygen derivatives, and other prooxidants play an essential role in the formation of essential components and biological activation of essential components. However, at the same time, free radicals are toxic and can cause cellular damage through the oxidation of lipids, proteins, and DNA. Besides, immune cell function can also be interrupted by the existence of free radical activity. One of the substances that minimize the danger of free radicals is antioxidants.

According to Safitri (2011), Antioxidants help free radical inactivation. Some of the potent antioxidants in food are not expressed as essential nutrients. Such compounds include carotenoids, flavonoids, phenols, and Polyphenols (Webb, 2007 in Safitri, 2011). One of the most widely encountered groups of phenol compounds is flavonoids. Flavonoids and other phenol compounds have a wide range of biological benefits, including antioxidants, anti-inflammatory, inhibiting microbial growth, and preventing tumor onset (Miguel, 2011). Compound flavonoids and phenolic acid, in vitro, proved potentially as an antioxidant.

Anthocyanins are anti-inflammatory and antiseptic substances that function to inhibit mutations due to mutagen derived from cooked foods and suppress the polyferation of cancer cells. According to Miguel (2011), Anthocyanins are highly reactive to ROS/RNS due to the electron deficiency of ROS/RNS whereas when viewed from the relationship between the structure and the reduction potential indicating that the 3-hydroxyl group (ring C), this glycosylation reduces the activity as an antioxidant. Anthocyanins on Java Plum extract is thought to be able to increase the antioxidant enzymes such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR) and Menscavenger superoxide and Peroksinitrit. Various physiological activities of anthocyanins can have a significant impact on preventing cancer, diabetes, as well as cardiovascular and neurological diseases. It is supported by MacDougall Research (2002), which mentions that anthocyanins have anti-allergic and antithrombotic benefits.

The study reported that before Java Plum has displayed on the Model HUVECs preeclampsia, it did not do a phytochemical test to find out the type and the active compounds on the Java plum ethanol extract. Therefore, this study has not been able to mention the content of anthocyanins, flavonoids, and phenol compounds in the Java plum extract. Nevertheless, according to Safitri (2011), based on the characteristics of the dark purple ethanol extract of Java plum has a content of anthocyanins 12,16 ± 0.08 mg/g (frozen dried fruit). According to Sari (2009), Java Plum Fruit has a higher anthocyanin content of 161 mg/100g compared with Strawberry (97MG/100g) and red wine (88mg/100g) and according to Lestario (2003) antioxidant activity 64,75 ± 0.11%.

CONCLUSION

Administering the Java plum extract at a dose of 100 ppm, 200 ppm, and 400 ppm can decrease the expression of ICAM-1 on the Model HUVECs preeclampsia. It is necessary to do the phytochemical test to determine the standard anthocyanin on the Java plum extract to reduce the expression of ICAM-1.

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