

Red Pomegranate Extracts on Catalase Levels in Huvecs Culture Which are Exposed Preeclampsia Plasma

Januarsih

Midwifery Departement Poltekkes Kemenkes Banjarmasin
Mistar Cokrokusumo Street 1A Banjarbaru Indonesia
Email: januarsih.januarsih@gmail.com
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Abstract: In a normal pregnancy, there is an increase in the production of free radicals, compared to on not pregnant and when the preeclampsia occurs the production is even more. This situation controlled with anti-oxidants. In general, antioxidants divided into two groups, namely: enzymatic antioxidants / primary antioxidants / antioxidant deterrents and nonenzymatic antioxidants consist of SOD, GPx and CAT. The purpose of this study is to determine the effect of giving Pomegranate Fruit extract to CAT levels in HUVECs who exposed to plasma preeclampsia. This study used a laboratory exploration method with samples: 1 negative control group (HUVECs culture exposed to normal 2% plasma pregnancy). One positive control group (HUVECs culture exposed to 2% preeclamptic plasma) and three treatment groups (HUVECs culture exposed to plasma 2% preeclampsia was given red pomegranate extract at different doses of 14 ppm, 28 ppm, 56 ppm, followed by calculating the amount of CAT which was a marker of intracellular antioxidants. Using the assumption of normality and homogeneity of variance tests that was using the Levene test as a prerequisite for parametric statistical testing, (2) One Way ANOVA Test (F Test), and (3) Pearson correlation test. The result is Red Pomegranate extract can prevent a decrease in CAT levels at a dose of 56 ppm. In conclusion, the Pomegranate Fruit extract can increase CAT levels

Keywords: Red pomegranate; Catalase; Preeclampsia

INTRODUCTION

Preeclampsia is part of hypertension in pregnancy and the cause of increasing maternal mortality. The prevalence of preeclampsia is between 3 - 8% of all pregnancies, whereas in developing countries the prevalence of preeclampsia is 9-25% of all pregnancies (Huang et al., 2013; Naljayan and Karumanchi, 2013; Polsani et al., 2013). Maternal mortality in Indonesia in 2011 was almost 30% due to preeclampsia (Ministry of Health Republic of Indonesia, 2013). The pathophysiology of preeclampsia is still unclear, so preeclampsia often referred to as the "disease of theory." George and Graner (2012) stated that the pathophysiology of preeclampsia is an increase in oxidative stress, decreased hemogynase, inflammation, immune adaptation, type 1 autoantibody, and genetic susceptibility.

The condition of oxidative stress controlled with anti-oxidants. Antioxidants them-

selves, some of which already contained in the body (endogenous) and some obtained from outside the body (exogenous). In general, antioxidants are divided into 2 groups, namely: enzymatic antioxidants / primary antioxidants / antioxidant deterrents and non-enzymatic antioxidants consisting of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (El Bahr., 2013).

Red Pomegranate has valuable compounds in various parts of the fruit, as well as functional and medical effects such as antioxidants, anticancer and anti-oxidative diseases that proven (Mousavinejad et al., 2009). Sugi-anto, (2011) states that the administration of red pomegranate juice can increase the levels of GPx in the blood of mice with maximum physical activity. Nishigaki (2008) stimulated HUVECs cultures exposed to 50 mM 1 ml of serum fetal bovine (GFBS) to increase lipid peroxidation and reduce antioxidant enzyme activity.

Nishigaki et al., (2008) stimulated HUVECs cultures exposed to 50 mM 1 ml of serum fetal bovine (GFBS) to increase lipid peroxidation and reduce antioxidant enzyme activity. But when given Red Pomegranate extract lipid peroxide was inhibited and there was an increase in the status of antioxidant enzymes (SOD, CAT, and GSHPx) in endothelial cells by suppressing ROS generation, thus limiting the interaction effect of AGE-RAGE.

Red Pomegranate contains phytochemicals which are rich in compounds that function as antioxidants or polyphenols, tannins and anthocyanins (Kholifa., 2010). Therefore, this study will aim to determine whether there is an effect of the administration of red pomegranate extract (*Punica granatum*) on increasing CAT levels in HUVECs cultures exposed to plasma preeclampsia.

MATERIALS AND METHODS

Research design

The research design used in this study was experimental (real experimental) with a post test only control group design approach. In this study, treatment or intervention of the researcher was the culture of endothelial cells or Human Umbilical Vein Endothelial Cells (HUVECs) from umbilical placenta Newborns of women with normal pregnancy through childbirth SC without complications was exposed to the plasma of patients preeclampsia continued with the extract Pomegranate Red doses 14 ppm, 28 ppm and 56 ppm for the culture. While the phenomenon that occurs due to treatment or intervention from the researcher only observed after the procedure or intervention given in this study was the CAT content in HUVECs culture.

CAT examination used KIT with the principle of enzyme reaction with methanol containing H₂O₂. Formaldehyde that produced from this reaction quantitatively identified. The chromogen used was 4-amino-3 hydrazino-5-mercapto-1,2,4-triazole (Purpald) which could change the formaldehyde resultant from colorless to purple.

Population and Samples (Research Objectives).

The sample used in this study was endothelial cells originating from the umbilical cord of the newborn (BBL), which originated from maternity by Sectio Caesaria (SC) with-

out complications. The number of replications was 5, so the total number of samples became 30 observations. HUVECs were divided into 3 groups, namely: Group I: Negative control (K-) HUVECs + normal pregnancy plasma 2%, Group II: Positive control (K +) HUVECs + plasma preeclampsia 2%, Group III: Treatment 1 (P1) HUVECs + plasma preeclampsia 2% + Red Pomegranate extract 14 ppm, Group IV: Treatment 2 (P2) HUVECs + plasma preeclampsia 2% + Red Pomegranate extract 28 ppm, Group V: Treatment 3 (P3) HUVECs + plasma preeclampsia 2% + Red pomegranate extract 56 ppm.

This research is approved by ethics commission for research in the medical faculty of Brawijaya University, Indonesia.

Data Collection Technique

The sample in this study was endothelial cells originating from umbilicus newborns through SC labor that met the inclusion criteria: Healthy mothers with HB levels ≥ 10 g / dL with normal pregnancies and with SC labor and exclusion criteria: mothers with pregnancy complications. After delivery, the umbilicus was cut to 10cm, then inserted into the cord solution and stored in the refrigerator. The final step was to take endothelial cells through the umbilical vein by entering Collagenase solution. Then, the umbilical cord was warmed, using being held using our hands for 10 minutes (incubation). After that, Collagenase solution containing endothelial cells was removed from the center, by aspirating through a syringe that had been attached to the tip of the cannula and inserted into a 15 cc sterile centrifugation tube.

Then the center was rinsed with eight ccs PBS A solution. This is doing to clean the remaining endothelial cells in the center. After that, Collagenase solution containing endothelial cells removed from the umbilical cord by aspirating again through a syringe that had been attached to the tip of the cannula and inserted into a centrifugation tube which filled with Collagenase solution.

The solution containing the endothelial cells was centrifuged at a speed of 1000 rpm for 8 minutes so that a pellet containing endothelial cells obtained. Then the solution was transferred into a flask which was coated with 0.2% Gelatin solution and put into a 5% CO₂ incubator at 37 ° C for 30 minutes.

Data Analysis Techniques

In this study, data analysis techniques were carried out with three stages of calculation. There are three consecutive stages, namely: (1) normality assumption test using Shapiro-Wilk test and variance homogeneity using the Levene test as a prerequisite for testing parametric statistics, (2) One Way ANOVA test (F Test), and (3) test Pearson correlation. All calculations were carried out with the help of SPSS for Windows 19.0 software.

RESULT AND DISCUSSION

Red Pomegranate Extract effect on the levels of CAT

Table 1. CAT Level Testing with ANOVA and LSD 5%

Treatment	Mean \pm SD	p-value
K-	11.991 \pm 1.92 ^b	0.000
K+	5.290 \pm 0.538 ^a	
14 ppm	4.851 \pm 0.870 ^a	
28 ppm	6.692 \pm 1.666 ^a	
56 ppm	23.44 \pm 1.981 ^c	

Description: On average \pm sd if loading different letters means that there are significant differences ($p < 0.05$) and if loading the same letter means there is no significant difference ($p > 0.05$).

Table 2. Comparison of CAT Multiple Comparison with LSD 5%

Comparison		Average-Aaverage Difference	p-value
K-	K+	6.701	0.000
	14 ppm	7.140	0.000
	28 ppm	5.299	0.000
	56 ppm	-11.449	0.000
K+	14 ppm	0.439	0.651
	28 ppm	-1.402	0.158
	56 ppm	-18.150	0.000
14 ppm	28 ppm	-1.841	0.069
	56 ppm	-18.589	0.000
28 ppm	56 ppm	-16.748	0.000

Based on the table above, the comparison of the negative control group with positive control and all treatment groups, it was shown that the p-value in the K + group was less than 0.05 ($p < 0.05$). This means that there is a significant difference in the average CAT between the negative control group and K +.

This indicated that the administration of Red Pomegranate extract with a concentration of 56 ppm was able to increase CAT levels to higher levels than normal HUVECs culture groups. While the administration of Red Pomegranate extract at concentrations of 14 ppm and 28 ppm, has not been able to increase CAT levels significantly. This indicated by the average value \pm for the two groups containing the same letters as the positive control group.

Based on ANOVA test results using 5% LSD, the treatment of Red Pomegranate extract was significantly able to increase CAT levels to higher than HUVECs cultures with normal pregnant plasma. The results of this study indicate that Red Pomegranate extract does have antioxidant activity so that it can prevent a decrease in CAT levels in endothelial cells due to the presence of free radicals from plasma with 2% preeclampsia.

Antioxidants are substances or compounds that can slow or prevent the oxidation process, protect biological systems, counteract the potential effects of processes or reactions that cause excessive oxidation. The body has a free radical defense system in the form of enzymatic and non-enzymatic antioxidants — the enzymatic antioxidant system prepared by superoxide dismutase, catalase, and peroxidase which incorporated in the defense mechanism against ROS (El Bahr., 2013). Also, Emami et al., (2007) added some phenolic compounds (catechins, flavones, flavonols and isoflavones), tannins (ellagic acid, gallic acid, proanthocyanin), phenyl isopropanol (caffeine acid, coumaric acid and ferulic acid), lignans, catechol and many others are antioxidants.

Abimulyani et al., (2014) in an experimental study reported that endothelial cells exposed to plasma preeclampsia would increase lipid peroxidation levels in endothelial cells. However, when endothelial cells that presented to plasma preeclampsia given anthocyanin, there was a decrease in lipid peroxidation levels in the endothelial cells.

Fawole et al., (2011) stated that the anthocyanin content in red pomegranates fruit reaches 16.5-26.9 per 100 grams of juice. Anthocyanin is responsible for staining the red pomegranate. Miguel., (2011) reported that anthocyanin is a powerful antioxidant. Anthocyanins function as antioxidants in some ways: increasing the activity of antioxidant enzymes and electron donors for free radicals.

In the body, intracellular antioxidant enzymes consisting of SOD, CAT and Gpx function as the main defense lines in destroying free radicals. Red pomegranate extract can increase CAT levels according to the theory which states that in pathological conditions such as the formation of excessive amounts of free radicals, enzymes that function as endogenous antioxidants can decrease levels. Therefore, if there is an increase in free radicals in the body, exogenous antioxidants are needed to eliminate and neutralize the effects of free radicals (Astuti, 2008). So that in this study, the effective Red Pomegranate extract was able to prevent a decrease in CAT levels at a dose of 56 ppm.

CONCLUSION

Red Pomegranate extract (*Punica granatum*) has been shown to increase CAT levels in HUVECs cultures exposed to plasma preeclampsia.

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