ORIGINAL ARTICLE

Effect of Pravastatin on Levels of Malondealdehyde (MDA) And Endothelin-1 (ET-1) Preeclampsia Model Rats

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ABSTRACT

Introduction: Pravastatin is known to have a number of pleiotropic effects including reducing endothelial dysfunction, anti-inflammatory, antioxidants, conangiogenic, and antitrombotic. Pravastatin through the pleitropic effect is expected to be one of the alternative therapies to prevent preeclampsia. The limited strategy for prevention and treatment of preeclampsia is due to the unknown etiology and pathogenesis. These two markers are thought to contribute to the occurrence of preeclampsia although they cause it in two different pathways. MDA is a marker of oxidative stress as an end product of lipid peroxidation. ET-1 is a vasoconstrictor that plays a role in the pathogenesis of preeclampsia through increasing anti-angiogenic properties. Aim: to determine the effect of pravastatin on serum levels of MDA and ET-1 in preeclampsia rat models. **Methods:** This study consisted of 5 groups; negative control/ K(-) consisted of normal pregnant rats, positive control/ K(+) consisted of rat model of preeclampsia (rat model of preeclampsia induced by administration of L-NAME at a dose of 125 mg/kg BW/day since gestational age 13-19 days), treatment groups 1, 2, and 3 (rat model of preeclampsia given pravastatin with 3 different doses; 2 mg/day (P1), 4 mg/day (P2) and 8 mg/day(P3)) at 13-19 days of gestation. The rat model of preeclampsia was determined based on blood pressure > 140/90 with urine protein > +1. After termination, blood was drawn to measure serum MDA and ET-1 levels. **Results:** Serum levels of MDA and ET-1 were decreased in groups P2 and P3 compared to groups K(+). Statistically, there was a significant difference in the mean levels of MDA (p=0.001) and ET-1 (p=0.000) between each group. **Conclusion:** Pravastatin can prevent preeclampsia by decreasing MDA and ET-1.
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Keywords: Preeclampsia, Pravastatin, MDA, ET-1, Nitric oxide

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INTRODUCTION

Preeclampsia is a pregnancy-specific clinical condition that appears after 20 weeks of gestation (1). Preeclampsia is characterized by the onset of hypertension, proteinuria, hematological complications, and uteroplacental disorders (1,2). Preeclampsia is classified into early-onset (<33 weeks) and late-onset (>34 weeks) (3). The primary etiology responsible for the onset of preeclampsia is not known with certainty. Still, some literature states that preeclampsia is a combination of several processes, including impaired cytotrophoblast invasion, placental ischemia, and oxidative stress that causes maternal vascular endothelial dysfunction (4–6).

Oxidative stress is thought to have an essential role in the development of preeclampsia (2,6). Oxidative stress is characterized by an imbalance of endogenous antioxidant compounds and the formation of free radicals that lead to lipid membrane damage and lipid peroxide production (2,5,7). The end product of lipid peroxidation is Malondialdehyde (MDA), which is highly toxic, potentially mutagenic, and atherogenic (6,8). Several studies have reported increased MDA in serum, plasma, and placental tissue samples from pregnant women with preeclampsia (9–13).

In addition to oxidative stress, an increase of vasoconstrictor agents is also thought to be the cause of preeclampsia. Endothelin-1 (ET-1) is a major endothelium-derived vasoconstrictor that can play a

role in the pathogenesis of preeclampsia through the induction of trophoblast cell (TC) apoptosis and an increase in oxidant and anti-angiogenic substances (14). Impaired spiral artery remodeling can trigger placental hypoxia, stimulate the release of imbalance factors such as ET as a potent vasoconstrictor, and cause impaired vascular hemostasis, causing constriction of blood vessels (14).

Prevention and treatment of preeclampsia are still limited (15,16). Statins have several pleiotropic effects, including reducing endothelial dysfunction, anti-inflammatory, antioxidant, proangiogenic, and antithrombotic by increasing the expression of HMOX-1, thereby inhibiting HMG-CoA (17). One of its derivatives, pravastatin, has been shown to increase eNOS activity in vitro and in vivo, resulting in overexpression of the vasodilating factor NO (18,19). As an antioxidant, pravastatin can upregulate endogenous antioxidant pathways (20).

The study of effect of pravastatin as prevention and treatment of preeclampsia is still very limited as it is considered quite difficult if the pathomechanism and causal relationship are studied directly in humans. The use of supplements and drugs for the prevention and treatment of preeclampsia has been carried out, but with limited success (1,21). Pravastatin is assumed to have antioxidant and antiangiogenic effects that can improve conditions of oxidative stress and improve endothelial dysfunction that occurs in preeclampsia. To prove this assumption, a study was conducted on the effect of pravastatin on MDA and ET-1 levels in preeclampsia rat model animals. Induction of preeclampsia using injection of Nitro L-Arginine Methyl Ester (L-NAME). L-NAME is an inhibitor of nitric oxide synthase (NOS). NOS inhibition can increase blood pressure, vasoconstriction, and increase oxidative stress, and also cause proteinuria which resembles the characteristics of preeclampsia (22,23). This research is expected to be a reference for the use of pravastatin as an alternative therapy for preeclampsia in humans through various pathomechanisms of preeclampsia.

MATERIALS AND METHODS

Experimental Animal Model

This type of research was a true experiment with a posttest only control group approach conducted in vivo on Wistar rats. In this study, the population used pregnant rat Wistar strain. We determined pregnancy using indicators of weight gain of rat. The rat's body weight was measured after matting and it was then measured again on the 7th and 12th day after matting. Pregnant rat would experience weight gain around 15-20 grams in 12 days. We did not use a vaginal plug because it is a sign of copulation but not necessarily a sign of pregnancy. The first day of pregnancy was determined at 1x24 hours after matting. We also used physical examination (inspection and palpation) on the abdomen of rats from 10 days of gestation. We used rats that had never been pregnant before to facilitate physical examination. Pregnant rats have wider stomachs. However, this examination is subjective.

The number of samples was 20 pregnant rats which were divided into 5 groups. The negative control/ K(-) consisted of normal pregnant rats, positive control/ K(+) consisted of rat model of preeclampsia (induced by administration of L-NAME at a dose of 125 mg/kg BW/ day since gestational age 13-19 days), treatment groups P1, P2, and P3 consisted of rat model of preeclampsia given pravastatin with 3 different doses; 2 mg/day (P1), 4 mg/day (P2), and 8 mg/day (P3) at 13-19 days of gestation. Termination was carried out on the 19th day of pregnancy by cervical dislocation method (24).

The samples taken were serum MDA and Endothelin-1. The procedure carried out on experimental animal models has previously received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya Malang, Indonesia (Code of Ethics: 13/EC/KEPK/01/2021).

Induction of Preeclampsia

This was the manufacture of animal models of preeclampsia by injecting L-NAME (C7H=N5O4.HCl) Cayman Chemical brand CAS registry no. 51298-62-5 as a NOS inhibitor as much as 125 mg/kg/BW/day intraperitoneally in pregnant rats from day 13 to day 19 of pregnancy. The success of induction of preeclampsia was determined based on an increase in blood pressure > 140/90 based on the results of blood pressure measurements using Tail Cuff Method by CODA and found proteinuria >+1.

Administration of Pravastatin

Pravastatin was given orally using a probe at a dose of 2 mg/day, 4 mg/day, and 8 mg/day from day 13 to day 19 of pregnancy. The dose of pravastatin refers to a previous study that used pravastatin 10 mg, 20 mg, and 40 mg in humans, then the dose was converted according to the body weight of the rat (25). Pravastatin was given for 7 days of pregnancy start from day 13 to day 19 of pregnancy.

Blood Pressure Measurement

Blood pressure measurement was carried out in the Physiology Laboratory of the Faculty of Medicine, Universitas Brawijaya Malang, using the Tail Cuff method using the Kent scientific CODA tool. Blood pressure measurements were carried out on the 12th, 15th, and 19th days of pregnancy. Measurement of blood pressure on 12th of pregnancy was to ensure all samples have normal blood pressure. Measurement of blood pressure on the 15th day of pregnancy was to ensure the successful induction of L-NAME in preeclampsia rat model which was considered equivalent in the 2nd trimester of gestation in humans. Meanwhile, the measurement of

blood pressure on day-19 was to measure the success of pravastatin administration in rat models of preeclampsia which was considered equivalent to the 3rd trimester of pregnancy in humans (26).

Urine Protein Measurement

Rats were put into metabolite cages for 1x24 hours to collect their urine. Urine protein was measured using the urinalysis reagent test strips method on the 12th, 15th, and 19th day of pregnancy. The results of urine protein in qualitative data were converted into semi-quantitative data. Urine protein 0.3 g/L is equivalent to (+), urine protein 1.0 g/L is equivalent to (++), urine protein 3.0 g/L is equivalent to (+++), and urine protein >10 g /L is equivalent to (++++). Urine protein data for each group was calculated on average.

Measurement of MDA and ET-1 Serum Levels

Serum levels of MDA and ET-1 were measured by the ELISA method with Catalog No. E-EL-0060 (MDA) and Catalog No. E-EL-R1458 (ET-1) by Elabscience.

Data Analysis

Data analysis with SPSS 25.00. One-way ANOVA test was used to measure the average levels between groups of observations. LSD test was used to determine the dose that had the most effect on MDA and ET-1 levels. Correlation test was used to determine the dose effect of pravastatin on serum MDA and ET-1 levels.

RESULTS

Identification of The Results of Making Animal Models of Preeclampsia

L-NAME 125 mg/Kg/day was administered intraperitoneally since the 13th day of gestation resulted in pregnant Wistar rats (*Rattus norvegicus*) with clinical signs resembling preeclampsia. These clinical signs were marked by an increase in blood pressure > 140/90 and urine protein > +1 in the K(+) group from the 13th to the 19th day of pregnancy. However, we were able to notice elevated blood pressure and find proteinuria in the K(+) group on the 15th day of gestation when blood pressure and proteinuria were measured again. The following is the results of the average systolic blood pressure and urine protein in rats that have been given 125 mg/day of L-NAME injection (table I).

The mean systolic blood pressure and proteinuria of pregnant rat model of preeclampsia were measured at 12th, 15th, and 19th gestations. The mean systolic blood pressure increased and we found urine protein in pregnant rats given L-NAME injection of 125 mg/kg BW/day. We just noticed an increase blood pressure and found proteinuria since 15th (G15) gestation when blood pressure and urine protein was measured again. The highest blood pressure and urine protein were experienced by the K(+) group. At 19th (G19) gestasion, blood pressure and proteinuria continued to increase in the K(+) group, but blood pressure and proteinuria in groups P1, P2, and P3 (the group of rat model of preeclampsia given pravastatin) decreased with increasing pravastatin dose. Of the three groups of preeclampsia rat models which were given pravastatin, the P3 group had the lowest blood pressure and urine protein. Blood pressure and proteinuria in the P3 group were almost close to the values of the negative control group/ K(-).

Effects of Pravastatin on MDA Serum Levels

The parameter test results with one-way ANOVA in the five groups of observations n=4 showed that the mean MDA levels were statistically significant ($p=0.001<\alpha$). The highest mean MDA level was seen in the K(+) group. The results of the LSD test showed that there was a significant difference in the mean MDA levels of K(+) and K(-) ($p=0.000< \alpha$). The mean MDA levels were significantly different in groups P2 ($p=0.011<\alpha$) and P3 ($p=0.0000<\alpha$) when compared to group K(+). At the same time, the correlation between dose and MDA levels showed a non-significant correlation because the p-value = 0.057. So, it can be concluded that there is no correlation between increasing pravastatin dose and decreasing MDA levels (table II).

Effects of Pravastatin on ET-1 Serum Levels

Based on the one-way ANOVA test in all groups of observations with each group n=4, the results were $p=0.000<\alpha$. The highest mean ET-1 level was in the K(+) group, and the lowest was in the K(-) group. Based on the LSD test, it was found that there was a significant difference between the positive control group and the

Table I: Results of Systolic Blood Pressure dan Urine Protein Measurements in Preeclampsia Model Pregnant Rats Given Pravastatin with Different Doses

Group	Systolic Blood Pressure (mm/Hg)			Urine Protein (g/L)		
	G12	G15	G19	G12	G15	G19
Negative control/ K(-)	123.67± 2.91	123.22± 3.73	120.78±7.46	Negative	Negative	Negative
Positive control (L-NAME 125 mg/kgBW/day)/ K(+)	119.08±10.70	159.32±18.88	177.14±23.92	Negative	1.0	3.0
L-NAME+pravastatin 2 mg/day (P1)	114.86±14.66	150.36±6.54	135.36±3.75	Negative	1.0	0.475
L-NAME+pravastatin 4 mg/day (P2)	117±9.66	148.45±8.50	129.68±6.78	Negative	0.65	0.2
L-NAME+pravastatin 8 mg/day (P2)	114.82±10.20	143.59± 8.47	120.05±7.25	Negative	0.3	0.18

The mean systolic blood pressure (mmHg) dan urine protein (g/L) of pregnant rat model of preeclampsia were measured at the 12th (G12). 15th (G15). and 19th(G19) gestation. K(-) is a group of normal pregnant rats. K(+) is a pregnant rat model of preeclampsia. P1. P2. and P3 are rat model of preeclampsia given pravastatin at a dose of 2 mg/day. 4 mg/day. and 8 mg/day.

Table II: Mean Levels of MDA in Pregnant Rat Model of Preeclampsia

Group	Mean ± SD	p-value
Negative control/ K(-)	21.74±1.65[a]	0,001
Positive control (L-NAME 125 mg/kgBW/ day)/ K(+)	32.19±0.44[c]	
L-NAME+pravastatin 2 mg/day (P1)	27.70±6.19[bc]	
L-NAME+pravastatin 4 mg/day (P2)	25.87±4.09[ab]	
L-NAME+pravastatin 8 mg/day (P2)	21.77±4.63[a]	

The results of the one-way ANOVA test show that there is a significant difference if the p -value is <0.05, and there is no significant difference if the p-value is >0.05. In the LSD test results, if the mean ± SD contains different letters in the comparison of the two groups, it can be interpreted that there is a significant difference between the two groups (p -value <0.05) and vice versa. K(-) is a group of normal pregnant rats, K(+) is a pregnant rat model of pre-eclampsia, P1, P2, and P3 are rat model of a preeclampsia given pravastatin of 2 mg/day, 4 mg/day, and 8 mg/day.

negative control group (p = 0.000 <α). The decrease in ET-1 levels appeared to be in line with the increase in dose in the pravastatin group given P1, P2, and P3. The most effective amount in this study based on the LSD test was at a dose of 4 mg/day and 8 mg/day (p=0.000 <α.). The correlation between dose and ET-1 levels showed a strong relationship but in the opposite direction p= 0.006 (r=-739). This means that the higher the pravastatin dose, the lower the ET-1 level (table III).

Table III: Mean Levels of ET-1 in Pregnant Rat Model of Preeclampsia

Group	Mean ± SD	p-value
Negative control/ K(-)	3.50±0.52[b]	0.000
Positive control (L-NAME 125 mg/kgBW/ day)/ K(+)	13.71 +1.30[a]	
L-NAME+pravastatin 2 mg/day (P1)	11.96± 3.05[a]	
L-NAME+pravastatin 4 mg/day (P2)	4.732± 2.54[b]	
L-NAME+pravastatin 8 mg/day (P2)	3.67±0.29[b]	

The results of the one-way ANOVA test show that there is a significant difference if the p -value is <0.05, and there is no significant difference if the p-value is >0.05. In the LSD test results, if the mean ± SD contains different letters in the comparison of the two groups, it can be interpreted that there is a significant difference between the two groups (p -value <0.05) and vice versa. K(-) is a group of normal pregnant rats, K(+) is a pregnant rat model of pre-eclampsia, P1, P2, and P3 are rat model of a preeclampsia given pravastatin of 2 mg/day, 4 mg/day, and 8 mg/day.

DISCUSSION

In this study, we demonstrated the description of preeclampsia using an animal model of preeclampsia induced by injection of L-NAME Pravastatin (statin), which is hydrophilic and able to improve endothelial dysfunction in animal models of preeclampsia by decreasing levels of MDA and ET-1.

The etiology of preeclampsia is still not fully understood, but the role of the placenta is considered to be the main etiology in the process of preeclampsia (27). The placenta that is experiencing hypoxia continuously will stimulate the release of imbalance factors such as ET as a potent vasoconstrictor causing vascular hemostasis disorders, causing constriction of blood vessels. Stage two of preeclampsia occurs when the release of vasoactive factors during the first stage causes endothelial dysfunction (14). Placental ischemia induces ET-1 expression by increasing circulating sFlt-1, which directly inhibits VEGF and ultimately contributes to the development of preeclampsia (28). The role of ET-1 in hypertension is not yet clear genetically but it is assumed that the presence of ET-1 in serum is due to the blockade of eNOS by L-NAME (29). The role of ET-1 in preeclampsia is actually quite complicated because it is also related to the presence of VEGF. In this paper, we do not present the results of VEGF but a decrease in VEGF will contribute to the increase in sFlt associated with inhibition of eNOS so that ET-1 is increased (30). ET-1 is an endothelium peptide derivative that is vasoconstrictive by binding to the G-protein receptor (ETA or ETB). In preeclampsia, ET-1 is activated by an inducer factor in the blood vessels via the ETA receptor in conditions of placental ischemia. We induced preeclampsia at stage two because we induced preeclampsia with L-NAME at a time when placentation was thought to be complete. Meanwhile, stage one of preeclampsia begins when the trophoblast invasion fails.

We used experimental animals induced by injection of L-NAME 125 mg/Kg/day to examine the pleiotropic effect of pravastatin in ameliorating endothelial dysfunction. The results showed that a pathophysiology resembles the picture of preeclampsia in rats, namely an increase in systolic blood pressure and urine protein. The indicators used to diagnose preeclampsia were blood pressure and urine protein in this study. No other clinical signs and symptoms were found in sample. The increase in blood pressure after administration of L-NAME is related to the working principle of L-NAME, namely the occurrence of eNOS uncoupling with a decrease in NO character and an increase in superoxide anion (O2-) (31). The presence of urinary protein in preeclampsia is associated with endothelial dysfunction resulting in glomerular damage and an increase in the size of protein permeability across the glomerulus (32). We also observed an increase in ET-1 levels in a group of animal models of preeclampsia. ET-1 mediates the balance of vascular tone. An equilibrium is reached if vasodilation conditions will stimulate normal blood pressure regulation during pregnancy. Still, if there is a deviation from this receptor, it will lead to vasoconstriction and hypertension, thereby triggering preeclampsia (28).

The dose of L-NAME given in this study refers to the previous research, namely injection of L-NAME 125 mg/Kg/day from day 13 of pregnancy to day 21/22 of gestation can increase systolic blood pressure in rats (33). The success of making animal models of preeclampsia depends on the dose, time of administration, duration, and method of administration in experimental animals (31). In this study, pravastatin at a dose of 4 mg/day and 8 mg/day reduced ET-1 and MDA levels in rat (Rattus norvegicus) model of preeclampsia. Human studies with pravastatin 40 mg/day (equivalent to 8 mg/day in animals) in women with clinical signs of preeclampsia at 24-29 weeks of gestation successfully lowered systolic blood pressure followed by stabilization of sFlt-1, sEng,

and ET- 1 (34). This finding is in line with the results of a previous study that showed pravastatin at a dose of 5 mg/day (equivalent to 20 mg/day in humans) given to CD-1 mice given on days 9 to 18 of gestation showed promising results. Decreased sFlt-1 and sEng were followed by upregulation of VEGF and PlGF and decreased hypoxia markers (35). Through inhibition of HMG-CoA reductase, pravastatin can provide a pleiotropic effect by reducing cellular cholesterol synthesis and isoprenoid levels, which significantly contribute to endothelial cells to increase NO bioavailability. The availability of NO will inhibit sFlt-1 and sEng to increase VEGF and PlGF for endothelial repair through a decrease in ET-1 (18). Pravastatin restores NOS function under pathological conditions, increases tissue-type plasminogen activator expression, and decreases expression of the potent vasoconstrictor ET-1 (34). This study also showed that a dose of 2 mg/day could not reduce levels of ET-1 in the preeclampsia rat model. We assume that the condition is influenced by hypoxia which is a potent inducer of ET expression via HIF-1-α, resulting in a low oxygen environment, abnormal trophoblast invasion, and impaired spiral artery remodeling (36).

This study found that preeclampsia rat models had higher MDA levels than normal pregnant rats. Preeclampsia can cause increased levels of MDA as an end product of lipid peroxidation. The administration of L-NAME causes a decrease in NO production which leads to the formation of uncoupled eNOS, which affects increasing the production of superoxide ions. In addition, administration of L-NAME activates iNOS generating large amounts of NO, which rapidly binds to superoxide ions to produce peroxynitrite ($OONO^-$). Peroxynitrite is a potent oxidizing agent to form lipid peroxides (37). Lipid peroxide has a role in damaging the membranes, nucleus, and proteins of endothelial cells. The final product of lipid peroxide is MDA, and in this study, it was used as a significant marker of oxidative stress. The high level of MDA in the group of rats injected with L-NAME was following several previous studies (9–13), which stated that there was a significant increase in serum MDA levels in patients with preeclampsia compared to normal pregnancies. Several other studies have shown that MDA levels increase in hypertensive conditions. Pregnant women with hypertension found an increase in MDA levels and decreased catalase activity (38).

In pregnant women with preeclampsia, lipoperoxidation products are increased. In addition, the availability of nitric oxide (NO) has decreased, especially in plasma and the placenta, which is due to an increase in nitric oxide synthase (NOS) activity. A superoxide anion can activate NO to produce a solid free-radical product, namely peroxynitrite anion (ONOO-). Excessive peroxynitrite levels cause the high synthesis of prostaglandins which are products of lipid peroxidation of arachidonic acid. These prostaglandins are responsible for vasoconstriction of blood vessels and trigger hypertension (39). Administration of pravastatin is considered able to reduce MDA levels. The doses of pravastatin that were supposed to reduce MDA levels significantly were doses of 4 mg/day and 8 mg/day. The results of the correlation test showed a significant relationship in the opposite direction, that is, the greater the dose of pravastatin, the lower the MDA level. The decrease in MDA levels after pravastatin administration was thought to be due to a reduction in ROS production, resulting in decreased free radicals that can oxidize lipids as an antioxidant effect. The antioxidant effect of statins acts in influencing NO synthesis. Early in the process, statins cause upregulation of the endothelial nitric-oxide synthase (eNOS) receptor, leading to eNOS being activated and modulating the mRNA stability of eNOS (40).

Pravastatin increases NO bioavailability and upregulates eNOS (41). Pravastatin also inhibits the expression of iNOS and reduces renal MDA levels (42). Increased regulation of eNOS and decreased expression of iNOS caused a decrease in the level of NO bound to superoxide so that peroxynitrite production also decreased. The decreased peroxynitrite is expected to reduce lipid peroxidation, leading to MDA production as an end product. Brownfoot et al. in their research, stated that giving pravastatin increased cellular antioxidant response by controlling the expression of encoding genes, one of which was SOD (43). Pravastatin which has the effect of expanding NO bioavailability, prevents the formation of uncoupled eNOS and contributes to the reduction of superoxide. This condition can ultimately reduce oxidative stress that occurs in preeclampsia.

A limitation of our study is that we did not observe the effect of pravastatin in a group of normal pregnant rats. We also did not give L-NAME at the beginning of implantation and did not terminate at the time of early- and mid-gestation so that we were unable to distinguish the effect of pravastatin on early- and late-onset. In previous studies, early-onset and late-onset features will give a different picture in the pathophysiology of preeclampsia. The conclusion from our research is that pravastatin at a dose of 8mg/day/kgBW can improve preeclampsia by reducing ET-1 and MDA levels. Pravastatin is expected to be used as an alternative therapy for preeclampsia in humans, which of course through further researchs.

CONCLUSION

Allotment of pravastatin at a dose of 8 mg/day/kgBB leads to reduced serum levels of MDA and ET-1 in preeclampsia rat model at 13-19th gestation.

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