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Pravastatin Effect on soluble Fms-like tyrosine kinase-1 (sFlt-1), Tumor Necrosis Factor-α (TNF-α), Interleukin-10 (IL-10) Expression and Hepatocyte Cell Necrosis in The Liver Pregnant Rat Model of Preeclampsia

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ABSTRACT

Introduction: *Pravastatin is a reductase inhibitor that is able to inhibit HMG-CoA activity. Influences Heme Oxygenase-1 (HMOX-1) in producing CO and activating eNOS, thereby balancing angiogenic factors and reducing inflammation. This study objectives to determine the effect of pravastatin on the expression of tyrosine-1 such as soluble FMS (sFlt-1), Tumor Necrosis Factor-α (TNF-α), Interleukin-10 (IL-10), and the amount of hepatocyte cell necrosis in a pregnant rat model. preeclampsia.*

Method: *True experimental design with post test only control group design used liver tissue of wistar strain pregnant rats (Rattus norvegicus) as a preeclampsia model by administering pravastatin in 3 different doses.*

Results: *The expression of sFlt-1, TNF-α, and IL-10 as well as the number of hepatocyte cell necrosis in the five observation groups had a p value <α. Dose correlation with sFlt-1 expression (p-value 0.008; r = -0.576), TNF-α expression (p-value 0.001; r = -0.669), IL-10 expression (p-value 0.002; r = 0.644), number of hepatocyte cell necrosis (p-value 0.006; r = -0.591). Correlation of sFlt-1 and TNF-α expression (p-value 0.027; r = 0.594), sFlt-1 and IL-10 expression (p-value 0.007; r = -0.686), sFlt-1 expression with hepatocyte cell necrosis (p -value 0.034; r = 0.537), TNF-α expression and hepatocyte cell necrosis (p-value 0.049; r = 0.413), IL-10 expression, hepatocyte cell necrosis (p-value 0.045; r = -0.520).*

Conclusion: *Pravastatin is able to reduce the expression of sFlt-1, TNF-α and the amount of hepatocyte cell necrosis, as well as increasing the expression of IL-10. Significant results were found at a dose of 8 mg.*

Keywords – *Preeclampsia, Pravastatin, sFlt-1, TNF-α, IL-10, Hepar, Necrosis, Hepatocyte Cell*

1. INTRODUCTION

Preeclampsia is a condition characterized by an increase in systolic/diastolic blood pressure of 140/90 mmHg and proteinuria of 300 mg/hour in gestational age after 20 weeks with previous normal blood pressure[1]. The incidence of preeclampsia affects 2-30% of all pregnancies which can result in multi-organ damage such as renal insufficiency, hepatic complications, neurological, and hematologic[2]. The cause of preeclampsia is still unknown, but the pathophysiological mechanism of pregnancy with preeclampsia involves two phases, namely placental implantation disorders and maternal maternal syndrome which include angiogenesis imbalance, maternal endothelial dysfunction, oxidative stress, and increased pro-inflammatory cytokines, as well as decreased anti-inflammatory cytokines. These two pathways converge on endothelial dysfunction which causes hypertension, proteinuria, to organ damage[2,3].

The angiogenesis imbalance factor that occurs is one of them with an increase in soluble FMS-Like Tyrosine Kinase-1 (sFlt-1). sFlt-1 is a splice variant of Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1) and can bind and reduce levels of Vascular Endothelial Growth Factor (VEGF) and Placenta Growth Factor (PIGF) which play an important role in endothelial function and angiogenesis. Increased sFlt-1 and sEng can interfere with the regulation of Vegf and Plgf causing endothelial dysfunction, where sFlt-1 is a protein produced by the placenta in pregnancy. When endothelial dysfunction occurs in the placenta, it can increase Endothelin-1 (ET-1) which causes increased capillary permeability and vasoconstriction of blood vessels, causing the emergence of clinical maninfestation of preeclampsia to cause organ damage in pregnant women [4].

In addition to the occurrence of angiogenesis imbalance, preeclampsia can occur due to inflammation. Proinflammatory cytokines such as Interferon-ϒ, TNF-α, and IL-1,2, 6, 8,15,16, and 18 experienced an increase in circulation, while anti-inflammatory cytokines decreased. Tumor Necrosis Factor-α (TNF-α) is a proinflammatory cytokine made by macrophages, subcutaneous adipose tissue, and placenta where inflammatory cytokines are involved in liver inflammation by means of inflammation, proliferation, and apoptosis. TNF-α levels are elevated in women with preeclampsia and are associated with an increased risk of complications such as hemolysis, elevated liver enzyme, low platelet count (HELLP syndrome)[5,6]. When inflammation occurs, anti-inflammatory cytokines decrease, one of which is Interleukin-10 (IL-10) which has an important role during pregnancy because of its ability to inhibit the release of inflammatory cytokines Th1. This provides a balance in the control of inflammation at the mother-fetal interface (fetal-maternal interface)[7].

One of the organs damaged by preeclampsia is the liver. Hemorrhage may occur in peripheral lobe periportal cells causing cell necrosis and an increase in liver enzymes. Abnormal elevations in liver enzymes range from 20-30% of cases, with elevated levels of mild to moderate transaminases. While widespread bleeding is known as a subcapsular hematoma which can cause pain in the epigastric region and potentially cause liver rupture. Hepatic involvement consisting of hepatic artery vasospasm and fibrin precipitation in the portal and periportal areas of the hepatic lobules can lead to lobular ischemia and hepatocyte cell necrosis [8–10].

Necrosis of hepatocyte cells caused by preeclampsia occurs through placental endothelial dysfunction which is stimulated by an imbalance of angiogenesis factors and an increase in pro-inflammatory cytokines, as well as a decrease in anti-inflammatory cytokines where this will cause a decrease in blood flow to the liver resulting in endothelial damage to the liver blood vessels. Hepatic endothelial dysfunction can lead to decreased hepatic microcirculation and hepatocyte necrosis. The presence of endothelial dysfunction and decreased liver microcirculation is what can cause liver failure and liver rupture in preeclampsia conditions[2].

The use of low-dose aspirin and calcium supplementation are recommended as prevention of preeclampsia in women with a high risk of preeclampsia[11]. The use of low-dose aspirin is sometimes less effective in the prevention of preeclampsia, but if the dose of aspirin given too high during pregnancy can affect the mechanism of maternal hemostasis and increase the risk of postpartum bleeding, so currently pravastatin can be used as a preventive alternative in women with a high risk of preeclampsia[12].

Currently research related to pravastatin has often been carried out as a drug used as a prevention of preeclampsia with the content of first-generation statins, works by inhibiting the enzyme HMG-CoA reductase so as to reduce the amount of cholesterol produced by the body. Pravastatin has the ability to correct the angiogenesis imbalance of preeclampsia that is often associated with imbalances in the production of angiogenesis factors, such as increased sFlt-1 and decreased VEGF. Another benefit of giving pravastatin is that it can help reduce inflammation associated with preeclampsia by stopping activation of inflammatory pathways and reducing the number of proinflammatory cytokines as well as improving endothelial function, which can cause impaired blood flow and organ damage by reducing oxidative stress, increasing NO production, and inhibiting platelet aggregation[13].

2. OBJECTIVES

Research on pravastatin in the prevention of preeclampsia in pregnant women is still limited, so it needs to be done in experimental animals such as the effects on the liver organ that can occur damage due to preeclampsia. Pravastatin has the ability to correct imbalances in angiogenesis of preeclampsia which is often associated with imbalances in the production of angiogenesis factors and also reduces inflammation so as to prevent the occurrence of hepatocyte cell necrosis.

This study aims to determine the effect of pravastatin on the expression of sFlt-1, TNF-α, IL-10, and the number of hepatocyte cell necrosis. On the other hand, it is also to see the dose correlation that affects the expression of sFlt-1, TNF-α, IL-10, and the amount of hepatocyte cell necrosis. The study also looked at the relationship between the expression of sFlt-1, TNF-α, IL-10, and the amount of hepatocyte cell necrosis in preeclampsia conditions.

3. METHODS

This research design uses a true experimental design with a post test only control group design. The current research was carried out in vivo using biological material stored in the liver tissue of experimental pregnant rats (Rattus norvegicus) of the Wistar strain as a model of preeclampsia. Biological material stored from the liver tissue of experimental animals is stored at the Institute of Biosciences, Brawijaya University, Malang.

Experimental animals in this study used pregnant female rats (Rattus norvegicus) Wistar strain who were exposed to L-NAME with a minimum age of 14 weeks and body weight of 150-220 grams for this study and were given various doses of pravastatin which were divided into 5 groups, namely 1 group as a negative control group, 1 group as a positive control group, and 3 groups as a treatment group. The parameters observed were the expression of Soluble FMS-Like Tyrosine Kinase-1 (sFlt-1), Tumor Necrosis Factor-α (TNF-α), Interleukin-10 (IL-10), and the amount of hepatocyte cell necrosis in biological material stored in the liver organ.

Twenty five rats were included and grouped in 5. Pregnant rats with hypertension or that died before treatment, gave birth or died during the study were excluded. Meanwhile, damaged, dry and moldy paraffin block samples were not included in this study. The rats were then divided into 5 groups, namely:

- KN: Negative control (no treatment)
- KP: Positive control (125 mg/kg BW L-NAME)
- P1: 125 mg/kg BW L-NAME + 2mg/kg BW/day Pravastatin
- P2: 125mg/kg BW L-NAME + 4mg/kg BW/day Pravastatin
- P3: 125mg/kg BW L-NAME + 8mg/kg BW/day Pravastatin

Hepatic Tissue Cutting

The creation of hepatic tissue sample slides begins with a sample fixed with a 10% formalin buffer. Then the tissue is divided and cut to a thickness of 2 mm. Pieces of tissue are inserted in a tissue casset, labeled and closed. Furthermore, dehydration, clearing, impregnation or embedding, blocking, and cooling are carried out on the cooling plate. Sectioning is carried out with microtomes and the result of cutting is in the form of a thin tape of 3-5 µm. Then paraffin tape is inserted in a waterbath containing warm water ranging from 40 oC and taken with a poly-L-lysine slide. Then placed on a 40 oC hotplate overnight and stored at room temperature.

Expression of sFlt-1, TNF-α, and IL-10

Immunohistochemical staining begins one night incubation in the oven then deparafinization and blocking are carried out which are then stained using primary antibodies sFlt-1 (Anti sFlt-1 (sVEGFR-1) reiatech gmbh, cat#102- PA213) Reliatech Gmbh germany), TNF-α (Anti-TNF-α Antibody (52B83): sc-52746), and IL-10 (Anti-IL-10 Antibody (E-10): sc-8438) and continued with secondary antibodies, DAB administration and incubated, dehydration, and conterstained with mayer hematoxilin, Furthermore, mounting using Entellan until observations can be made.

Expression assessment was observed by double-blind method using Olympus BX53 microscope with 400x magnification and 5 µm bar scale at 10 field of view from all liver tissues, analyzed using ImageJ 1.53c software. The result obtained is that the percentage of all parts of liver tissue expressed in brown.

Number of Hepatocyte Cell Necrosis

Hematoxyline-Eosin (HE) staining is carried out after cutting slides using microtomes. Furthermore, the paraffin tape is deparafinized, soaking the hematoxylin solution, soaking the eosin solution, dried and given entellan as a cover until ready for observation Assessment of the number of hepatocyte cell necrosis was carried out by double-blind method using Olympus BX53 microscope with 400x magnification and 5 µm bar scale at 10 field of view. Each field of view counted the number of hepatocyte cells that underwent karyolysis necrosis, which is the final phase of necrosis which indicates significant damage characterized by the cell nucleus disappearing, appearing vaguely hollow.

Statistical Analysis

The analysis of the data used in this study was carried out in stages: 1) Test the normality of sample data; 2) Homogeneity test; 3) One Way ANOVA Difference Test; 4) Multiple comparison test; 5) Correlation test. All calculations are done using SPSS software for windows 19.

Ethics Statement

This research has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia.

4. RESULTS

sFlt-1 Expression in Rat Liver (Rattus norvegicus) Wistar Strain Model of Preeclampsia

The following are the results of *soluble FMS-like tyrosine kinase-1* (sFlt-1) expression analysis using the immunohistochemical method using Image J 1.53c software.

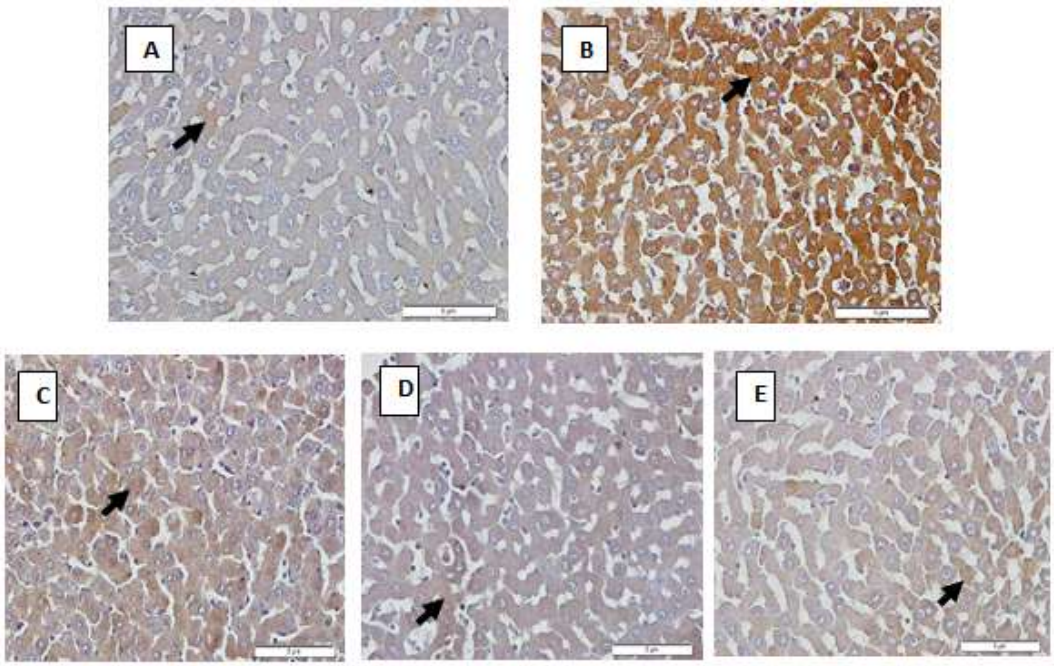

Figure 1: Histology of Differences in the Expression sFlt-1 in the Liver of Rattus norvegicus Preeclampsia Model

Note: Differences in sFlt-1 expression in Liver tissue between groups. The black arrow shows the expression

of sFlt-1 in the cytoplasm of brown Liver tissue seen from a microscope with a magnification of 400x and a scale bar of 5µm. (A) K (-) namely normal pregnant rat Liver; (B) K (+), namely the Liver of pregnant rat in the preeclampsia model; (C) P1, namely the Liver of a pregnant rat model of preeclampsia + pravastatin 2 mg/KgBW; (D) P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and (E) P3, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 8 mg/KgBW. The results of the percentage of sFlt-1 expression were then carried out by the One Way test.

Table 1: Effect of Pravastatin on sFlt-1 Expression in the Liver of Rattus norvegicus Preeclampsia Model

Research Group	(n)	Mean ± SD (Percentage)	*p-value* (*One Way ANOVA*)
Negative Control	5	14.35±5.78[a]	0.011
Positive Control	5	38.89±8.99[b]	
Treatment 1	5	26.08±8.37[ab]	
Treatment 2	5	20.10±16.62[ab]	
Treatment 3	5	16.47±10.09[a]	

***Note**: If the mean ± SD contains different letters (a and b) then there is a significant difference (p-value <0.05), whereas if it contains the same letters (a and ab or b and ab) then there is no significant difference (p -value >0.05). K (-) is the Liver of normal pregnant rat; K (+) is the Liver of pregnant rat in the preeclampsia model; P1 is the Liver of pregnant rats in the preeclampsia model + pravastatin 2 mg/KgBW; P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and P3, namely the Liver of pregnant rat in the preeclampsia model + pravastatin 8 mg/KgBW.*

TNF-α Expression in Rat Liver (Rattus norvegicus) Wistar Strain Model of Preeclampsia

The following are the results of *Tumor Necrosis Factor-α* (TNF-α) expression analysis using the immunohistochemical method using Image J 1.53c software.

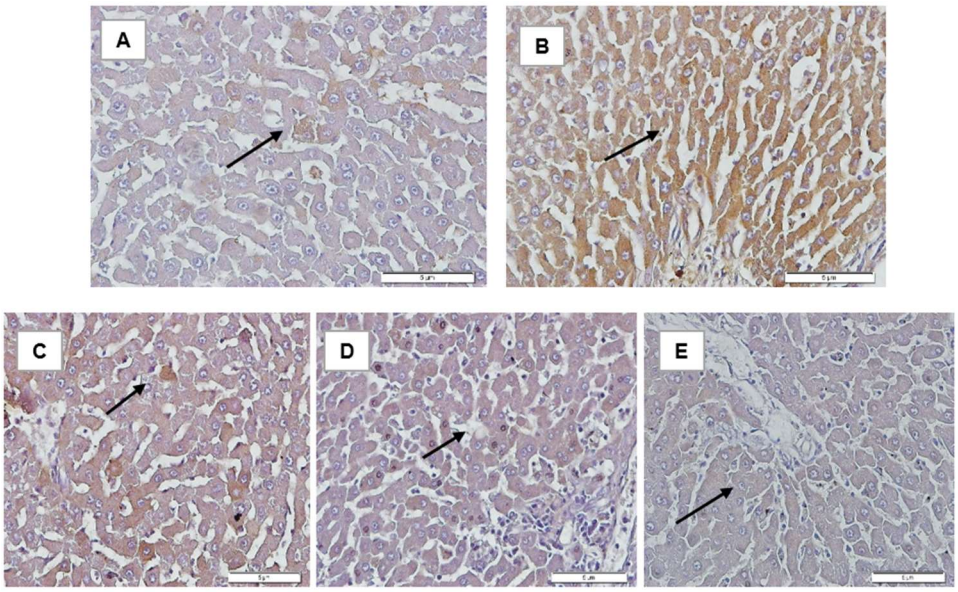

Figure 2: Histology of Differences in the Expression TNF-α in the Liver of Rattus norvegicus Preeclampsia Model

Note: Differences in TNF-α expression in Liver tissue between groups. The black arrow shows the expression of TNF-α in the of brown Liver tissue seen from a microscope with a magnification of 400x and a scale bar of 5µm. (A) K (-) namely normal pregnant rat Liver; (B) K (+), namely the Liver of pregnant rat in the preeclampsia model; (C) P1, namely the Liver of a pregnant rat model of preeclampsia + pravastatin 2

mg/KgBW; (D) P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and (E) P3, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 8 mg/KgBW. The results of the percentage of TNF-α expression were then carried out by the One Way test.

Table 2: Effect of Pravastatin on TNF-α Expression in the Liver of Rattus norvegicus Preeclampsia Model

Research Group	(n)	Mean ± SD (Percentage)	*p-value* (*One Way ANOVA*)
Negative Control	5	6.80±2.56[a]	
Positive Control	5	26.62±8.50[c]	
Treatment 1	5	21.43±8.43[bc]	0.002
Treatment 2	5	13.70±9.27[abc]	
Treatment 3	5	8.46±6.48[ab]	

***Note**: If the mean ± SD contains different letters (a and b) then there is a significant difference (p-value <0.05), whereas if it contains the same letters (a and ab or b and ab) then there is no significant difference (p -value >0.05). K (-) is the Liver of normal pregnant rat; K (+) is the Liver of pregnant rat in the preeclampsia model; P1 is the Liver of pregnant rats in the preeclampsia model + pravastatin 2 mg/KgBW; P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and P3, namely the Liver of pregnant rat in the preeclampsia model + pravastatin 8 mg/KgBW.*

IL-10 Expression in Rat Liver (Rattus norvegicus) Wistar Strain Model of Preeclampsia

The following are the results of *Interleukin-10* (IL-10) expression analysis using the immunohistochemical method using Image J 1.53c software.

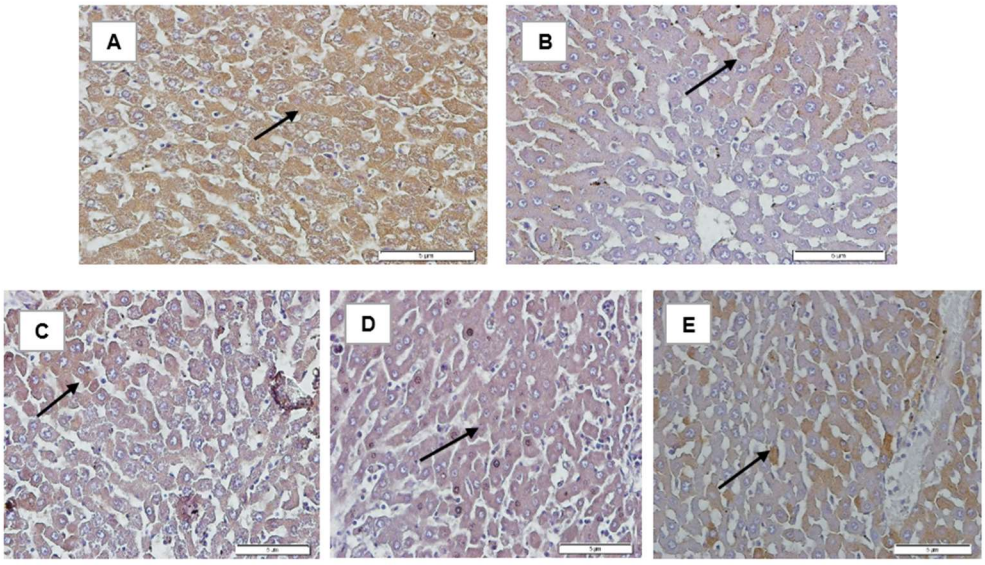

Figure 3: Histology of Differences in the Expression TNF-α in the Liver of Rattus norvegicus Preeclampsia Model

Note: Differences in IL-10 expression in Liver tissue between groups. The black arrow shows the expression of IL-10 in the of brown Liver tissue seen from a microscope with a magnification of 400x and a scale bar of 5µm. (A) K (-) namely normal pregnant rat Liver; (B) K (+), namely the Liver of pregnant rat in the preeclampsia model; (C) P1, namely the Liver of a pregnant rat model of preeclampsia + pravastatin 2 mg/KgBW; (D) P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and (E) P3, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 8 mg/KgBW. The results of the percentage of IL-10 expression were then carried out by the One Way test.

Table 2: Effect of Pravastatin on IL-10 Expression in the Liver of Rattus norvegicus Preeclampsia Model

Research Group	(n)	Mean ± SD (Percentage)	*p-value* (*One Way ANOVA*)
Negative Control	5	17.44±5.47[b]	
Positive Control	5	4.75±0.41[a]	
Treatment 1	5	11.84±3.09[ab]	0.011
Treatment 2	5	12.40±6.91[ab]	
Treatment 3	5	18.52±9.24[b]	

***Note**: If the mean ± SD contains different letters (a and b) then there is a significant difference (p-value <0.05), whereas if it contains the same letters (a and ab or b and ab) then there is no significant difference (p -value >0.05). K (-) is the Liver of normal pregnant rat; K (+) is the Liver of pregnant rat in the preeclampsia model; P1 is the Liver of pregnant rats in the preeclampsia model + pravastatin 2 mg/KgBW; P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and P3, namely the Liver of pregnant rat in the preeclampsia model + pravastatin 8 mg/KgBW.*

Hepatocyte Cell Necrosis in Rat Liver (Rattus norvegicus) Wistar Strain Model of Preeclampsia

The following are the results of the analysis of the amount of necrosis of liver hepatocyte cells using the Hematoxylin-Eosin method. The assessed necrosis is karyolysis necrosis.

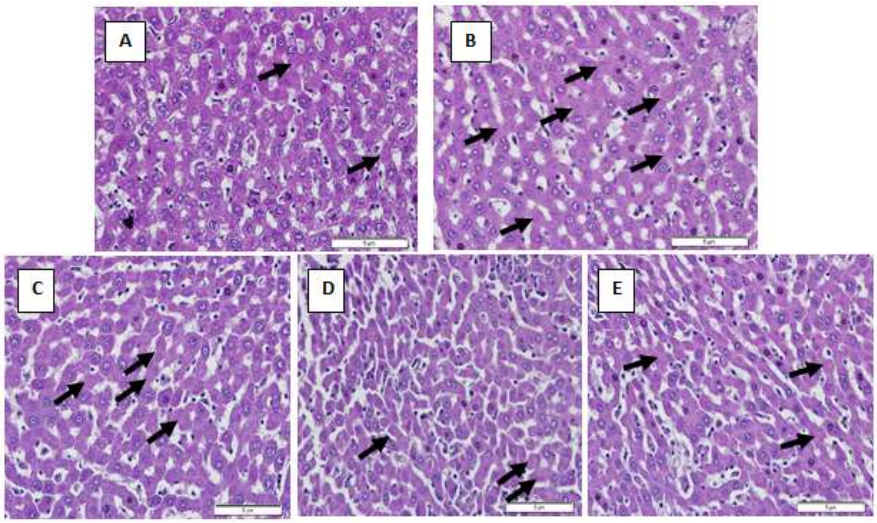

Figure 4: Histology of Differences in Necrosis of Hepatocyte Cells in Rat Liver (Rattus norvegicus) Wistar Strain Model of Preeclampsia

Note: Differences in hepatocyte cells that undergo necrosis in liver tissue between groups. Black arrows show cells undergoing karolysis necrosis seen from a microscope with a magnification of 400x and a bar scale of 5µm. (A) K (-) i.e. normal pregnant rat liver (mean 31.14); (B) K (+) i.e. liver rat bunting model of preeclampsia (mean 44.24); (C) P1 is a preeclampsia model of rat liver + pravastatin 2 mg/KgBW (mean 38.02); (D) P2 is a preeclampsia model of rat liver + pravastatin 4 mg/KgBW (mean 34.64); and (E) P3, namely the liver of pregnant rats model of preeclampsia + pravastatin 8 mg / KgBW (mean 31.52).

Table 4: Effect of Pravastatin on Necrosis of Hepatocyte Cells in the Liver of Rattus norvegicus Preeclampsia Model

Research Group	(n)	Mean ± SD (Number of Cells)	*p-value* (*One Way ANOVA*)
Negative Control	5	31.14±6.09[a]	0.026
Positive Control	5	44.24±8.03[b]	

Treatment 1	5	38.02±5.82[ab]	
Treatment 2	5	34.64±7.21[ab]	
Treatment 3	5	31.52±4.76[a]	

***Note**: If the mean ± SD contains different letters (a and b) then there is a significant difference (p-value <0.05), whereas if it contains the same letters (a and ab or b and ab) then there is no significant difference (p -value >0.05). K (-) is the Liver of normal pregnant rat; K (+) is the Liver of pregnant rat in the preeclampsia model; P1 is the Liver of pregnant rats in the preeclampsia model + pravastatin 2 mg/KgBW; P2, namely the Liver of pregnant rats in the preeclampsia model + pravastatin 4 mg/KgBW; and P3, namely the Liver of pregnant rat in the preeclampsia model + pravastatin 8 mg/KgBW.*

Table 5: Pravastatin Dose Correlation

Variables	Correlation coefficient (r)	*P-value*
Dose of Pravastatin on sFlt-1 expression	-0.576	0.008
Dose of Pravastatin on TNF-α expression	-0.669	0.001
Dose of Pravastatin on IL-10 expression	0.644	0.002
Dose of Pravastatin on Necrosis Hepatocyte Cells	-0.591	0.006

The Pearson correlation test showed that there was a significant correlation between the pravastatin dose variable and the variables sFlt-1 (p-value 0.008; r = -0.576), TNF-α expression (p-value 0.001; r = -0.669), IL-10 expression (p-value 0.002; r = 0.644), and necrosis hepatocyte cells (p-value 0.006; r=-0.591). The sFlt-1, TNF-α expression, and necrosis hepatocyte cells have a moderate correlation with a negative correlation towards pravastatin dose. The negative correlation between sFlt-1, TNF-α expression, and necrosis hepatocyte cells pravastatin indicates that the higher the dose of pravastatin, the more sFlt-1, TNF-α expression, and necrosis hepatocyte cells decreases. This means that the higher the dose, the lower the expression and the amount of cell necrosis of hepatocytes. Meanwhile, the positive correlation between IL-10 with pravastatin indicates that the higher the dose of pravastatin, the more IL-10 expression increases. IL-10 has a moderate correlation with a positive correlation direction to the dose of pravastatin. This means that the higher the dose, the more the expression increases.

Table 6: Correlation of sFlt-1, TNF- α, IL-10, and Necrosis Hepatocyte Cells

Variables	Correlation coefficient (r)	*P-value*
sFlt-1 expression on TNF-α expression	0.594	0.027
sFlt-1 expression on IL-10 expression	-0.686	0.007
sFlt-1 expression on Necrosis Hepatocyte Cells	0.537	0.034
TNF-α expression on Necrosis Hepatocyte Cells	0.413	0.049
IL-10 on Necrosis Hepatocyte Cells	-0.520	0.045

The Pearson correlation test showed that there was a significant correlation between sFlt-1 expression and TNF-α expression (p-value 0.027; r = 0.594), sFlt-1 expression with IL-10 expression (p-value 0.007; r = -0.686), sFlt-1 expression with hepatocyte cell necrosis (p-value 0.034; r = 0.537), TNF-α expression with hepatocyte cell necrosis (p-value 0.049; r = -0.413), and IL-10 expression with hepatocyte cell necrosis (p-value 0.045; r = -0.520). The expression of sFlt-1 with the expression of TNF-α and hepatocyte cell necrosis has a moderate correlation with a positive correlation, meaning that the increasing expression of sFlt-1, the expression of TNF-α and hepatocyte cell necrosis also increases significantly. While the expression of sFlt-1 with IL-10 expression has a moderate correlation with a negative correlation, meaning that the more increased the expression of sFlt-1, the lower the expression of IL-10. TNF-α expression with hepatocyte cell necrosis has a moderate correlation with a significant positive direction, meaning that increased TNF-α expression can increase the occurrence of hepatocyte cell necrosis, while IL-10 expression with hepatocyte cell necrosis has a moderate correlation with a negative direction, meaning that the lower IL-10 expression, the hepatocyte cell necrosis increases.

5. DISCUSSION

Preeclampsia is a condition characterized by systolic pressure hypertension >140 mmHg and diastolic>90 mmHg, in addition to proteinuria, dysfunction of other maternal organs (including liver, kidney, brain), or hematological involvement, and/or uteroplacental dysfunction[14]. Although preeclampsia is more than just gestational hypertension with proteinuria, the appearance of protein remains the main diagnostic criterion. It is an objective marker and reflects endothelial leakage throughout body systems that is characteristic of preeclampsia syndrome. Preeclampsia consists of two phases: abnormal placentation which is often influenced by genetic, environmental and immunological factors and the imbalance phase of angiogenesis, maternal endothelial dysfunction, oxidative stress, and increased placental inflammation[1,6,13,15,16].

The pathogenesis of preeclampsia is still not fully understood, but preeclampsia is associated with vascularization and implantation problems in the placenta that can create conditions of oxidative stress, inflammation, to angiogenesis imbalance. Many studies have shown that an increase in anti angiogenic biomarkers such as soluble FMS-like tyrosine kinase-1 (sFlt-1) has a role in the occurrence of preeclampsia[4]. sFLT-1 is a splice variant of the VEGF receptor similar to FMS-like tyrosine kinase-1 (Flt-1) that is sharply elevated in women with preeclampsia when compared to women with normotensive labor or women with normotensive preterm labor[17].

Sflt-1 is produced in the placenta during pregnancy in regulating the formation of blood vessels to support placental growth and fetal development. However, in preeclampsia pregnancies, Sflt-1 production is increased which can contribute to the pathogenesis of the disease. Most expression of sFLT-1 protein is localized in the syncytotrophoblast layer. sFlt-1 in the liver occurs through the process of gene transcription and translation of mRNA within the cell. The process of synthesis of the sFLT-1 protein begins with the transcription of the FLT-1 gene which produces the mRNA that encodes sFLT-1. This mRNA is then transported to ribosomes in the cytoplasm of the cell where the translational process occurs. During translation, ribosomes read the mRNA sequence and use that information to build sFLT-1 polypeptide chains that will eventually fold into functional protein forms[18–21].

This study proved that there was a change in liver condition in pregnant female preeclampsia rats given L-NAME induction 125 mg / KgBB. The value of liver sFlt-1 expression in the positive control group (given L-NAME induction 125 mg / KgBB) was shown to be higher when compared to the negative control group (pregnant rats not given L-NAME induction) with a p-value of $0.012<\alpha$. Administration of L-NAME 125 mg/KgBB was able to create a model of preeclampsia in rat and followed by a positive control group that had higher expression of sFlt-1 in the liver. This shows that it is true that preeclampsia can increase sFlt-1 levels and affect other organs, one of which is expressed in the liver.

In line with the research of Oe, et al. which aims to clarify liver function due to increased sFlt-1 in hypoxic conditions. Excessive amounts of sFlt-1 under hypoxic conditions can increase liver transaminase levels in plasma, inflammatory cells, and increase cytokine expression in the liver. This study states that sFlt-1 is expressed in the liver of preeclampsia rats (eNOS deficiency) using immunohistochemical methods. It can be concluded that the presence of preeclampsia with increased expression of sFlt-1 in the liver can also exacerbate oxidative stress and hypoxia, which is likely to cause severe liver damage[22].

In addition to increased anti-angiogenesis factors, preeclampsia is characterized by excessive and progressive activation of the immune system along with an increase in proinflammatory cytokines1 In this study proved that there were changes in the liver condition of pregnant female rats model of preeclampsia given L-NAME induction 125mg / KgBB. One of the changes that occurred was the expression of Tumor Necrosis Factor Alpha (TNF-α) detected in the liver using immunohistochemical methods.The value of TNF-α expression in the liver in the positive control group (given L-NAME induction 125mg/KgBB) was shown to be higher when compared to the negative control group with a p-value of $0.004 < \alpha$. This shows that it is true that the condition of preeclampsia can increase TNF-α levels and affect other organs where one of them is expressed in the liver.

This is in line with Alijotaz's research which states TNF-α can also affect endothelial function and cause

vasoconstriction which can worsen hypertension in preeclampsia and cause liver cell damage and liver dysfunction, so it can cause an increase in liver enzymes, a decrease in platelet count, and hemolytic anemia and Yang's research, et al mentioned that tumor necrosis factor-alpha (TNFα) is an inflammatory cytokine involved in liver inflammation. TNF-α provides inflammation, proliferation, and apoptosis[23,24].

In this study there was a significant difference in the average expression of IL-10 between the negative control group and the positive control group with a p-value of 0.021 < α. So it can be concluded that giving L-NAME 125 mg / kg BB can cause preeclampsia conditions which result in changes in the liver in the form of decreased IL-10 expression. This is in line with research conducted by4 Interleukin-10 (IL-10) is an anti-inflammatory cytokine mainly produced by Th2 cells, macrophages, NK cells, granulocytes, dendritic cells, and B cells that have been activated by autoantigens. IL-10 works to suppress inflammation and cellular immune responses while promoting humoral immune responses. In natural and adaptive immune cells, pro-inflammatory cytokines promote cell development and activation in the immune system. One of the most important properties of IL-10 is its anti-inflammatory action, which restrains the immune response under various stimuli. The balance between pro- and anti-inflammatory factors in the interaction of fetus and mother depends on interleukin-10. As a result, IL-10 is thought to be involved in the pathophysiology of preeclampsia. It is hypothesized that IL-10 levels in preeclampsia patients are lower than those of women who are normally pregnant[25].

Endothelial cell damage to preeclampsia conditions causes a decrease in blood flow in the liver where this causes endothelium in liver blood vessels to be damaged and there is a decrease in organ perfusion. Decreased perfusion of liver organs results in microcirculation (arterioles, venules, and capillaries) also decreased which should function to maintain the health and normal function of hepatocyte cells. When the microcirculation in the liver decreases, hypoxia can occur in its cells. In addition, the removal of residual products from liver cells will also be disrupted, which can cause oxidative stress and interfere with the normal function of hepatocytes. If the condition of decreased microcirculation in the liver continues to occur, it will increase hepatocellular damage conditions such as hepatocyte cell necrosis. Necrosis is a major pathway of cell death in many common injuries, such as ischemia, exposure to toxins, various infections, and trauma. In liver cells, it is usually characterized by changes in the cell nucleus (pycnotic nucleus, karyolysis, karyorexia) in the hepatocytes [15,26,27].

This study proved that there was a change in liver condition in pregnant female preeclampsia rats given L-NAME induction 125 mg / KgBB. The amount of liver hepatocyte cell necrosis in the positive control group (given L-NAME induction 125 mg/KgBW) was shown to be higher when compared to the negative control group with a p-value of 0.033 <α. Administration of L-NAME 125 mg / KgBB can increase the number of hepatocyte cell necrosis in the liver. This is in line with the research journal Situmorang., et al who gave L-NAME 70-80 mg/day showed results that had a greater fetal death effect on rat, higher blood pressure, and the amount of urine protein also increased. The L-NAME given group showed loss of polyhedral hepatocyte form, moderate hepatocyte hypertrophy, and sinusoidal dilatation. In this experimental model, proinflammatory mediators such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α) and prostaglandins released by these cells can facilitate inflammatory cell infiltration, as well as vacuolization and necrosis in liver cells[28].

Pravastatin is a naturally occurring statin produced by Aspergillus terreus and is type 1. Unlike other type 1 statins, pravastatin has a hydroxyl group at C6 which makes it hydrophilic. Its hydrophilic properties are reflected by the fact that pravastatin binds only to the polar head group of dodecylphosphocholine micelles (DPC), does not penetrate into the micelle core, and associates only with the hydrated membrane surface[29]. Pravastatin is a very potent and specific inhibitor of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. Once administered, pravastatin is absorbed rapidly and reaches peak plasma levels of the main component within one to two hours. The absolute bioavavivility of pravastatin is 17%, and its absorption in general is 34%[30]. Most of the malformations found in infants given lipophilic statins (61/64 [95%]) differed from hydrophilic statins such as pravastatin (3/64 [5%])[31].

Pravastatin is a prime candidate for preeclampsia prevention of several other statins. Pravastatin is a standard

and hydrosoluble type of statin. Pravastatin is an HMG-CoA reductase inhibitor that works by inhibiting the activity of HMG-CoA reductase which will affect Heme Oxygenase-1 (HMOX-1) in producing CO and activating eNOS so that NO production increases.Increased NO production can reduce ROS which also affects the reduction of oxidative stress to inflammation. In addition, increasing NO production can also balance angiogenic and inflammatory factors so that it can improve the condition of endothelial dysfunction while providing protection to the endothelium. When endothelial dysfunction decreases, blood flow in the liver will increase and reduce the condition of hepatocyte cell necrosis[13,32].

This study has proven that sFlt-1 expression, TNF-α expression, and liver hepatocyte cell necrosis in the pravastatin group, namely P1 and P2, were shown to be lower than in the positive control group given pravastatin doses, namely 2 and 4 mg / day. Meanwhile, the P3 group with preeclampsia model rat given a dose of pravastatin 8 mg/day was significantly lower when compared to the positive control group with p-value of sFlt-1 expression; TNF-α expression; hepatocyte cell necrosis (0.024; 0.008; 0.040<α). While the administration of pravastatin in groups P1, P2 and P3 found that IL-10 expression in liver organs increased, a significant difference in IL-10 expression between the positive control group and treatment group 3 with a p-value of 0.011<α The conclusion of the results of this study is the optimal dose to reduce sFlt-1 expression, TNF-α expression, and hepatocyte cell necrosis and increase IL-10 expression in the liver is 8 mg / kg BW / day.

The results of this study are in line with the research of de Alwis *et al* which states that the role of pravastatin administration significantly reduces the secretion of endothelin-1 (ET-1) and soluble FMS-like tyrosine kinase-1 (sFlt-1) which are the main mediators of endothelial dysfunction. Pravastatin in the study was also said to have no toxic effects, in contrast to rosuvastatin and simvastatin. In addition, a meta-analysis journal conducted by Mészáros *et al* stated that pravastatin administration can reduce the incidence of preeclampsia by 61% where there are 3 studies that give pravastatin at a dose of 40 mg daily for those who experience early-onset preeclampsia which can reduce sFlt-1 levels, have no side effects on the fetus or neonates when compared to the placebo group, so that it can provide benefits in stabilizing the mother's condition[33,34].

In addition, pravastatin increases the absorption of arginine microsomes, thereby inducing NO synthesis, which has a positive effect on microcirculation by directly inhibiting interferon-ϒ-mediated induction of MHC-II expression, leading to decreased T-cell activation. Through inhibition of T-cell activation and expression of adhesion molecules, statins reduce the immune system that releases inflammatory cell cytokines (monocytes, macrophages, lymphocytes) in the endothelium, so as to increase vascular reactivity and vasodilation, reduce hypertension to improve endothelial dysfunction due to preeclampsia which will certainly repair damaged endothelium of blood vessels to reduce hepatocyte cell necrosis. Pravastatin also works by regulating transcription and expression of HO-1 in endothelial and smooth muscle blood vessels; induction of HO-1 can lead to increased response of TGF-β, interleukin 10 (IL-10), and T lymphocytes[13,24,33].

6. CONCLUSSION

Administration of pravastatin can decrease the expression of sFlt-1 and TNF-α, increase IL-10 in the Hepar of Rattus norvegicus in a preeclampsia model. This impact shows a significant in P3 dose of 8mg/kgBW. Pravastatin has been shown to have a preventive effect in a mouse model of preeclampsia. Pravastatin is a potential candidate in the prevention or treatment of preeclampsia due to its good safety profile. Future research needs to evaluation the effect of pravastatin on the Hepar, which is very necessary to ensure the effectiveness and safety of using this drug in preeclampsia.

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