RESEARCH
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EFFECT OF PRAVASTATIN ON ARTERIOLE DIAMETER, GLOMERULAR AREA, INFLAMMATORY CELL NUMBER AND EPITHELIAL CELL NECROSIS IN MOUSE KIDNEYS WISTAR STRAIN (*RATTUS NOVERGICUS*) MODEL OF PREECLAMPSIA

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Abstract

Background: Preeclampsia is a hypertensive state in pregnancy (systolic > 140 mmHg and diastolic > 90 mmHg) and occurs at > 20 weeks' gestation. Preeclampsia is a disease that begins with dysfunction between the placenta and the mother, causing oxidative stress, endothelial dysfunction, hypoxia and systemic inflammatory responses that affect various systems and organs, one of which is the kidneys. The kidneys will experience decreased function due to angiogenic imbalance resulting in vascular vasoconstriction which results in a decrease in Glomerular Filtration Rate (GFR) causing a decrease in impaired glomerular perfusion and causing cell hypoxia thus triggering maternal oxidative stress increasing Reactive Oxygen Species (ROS) resulting in inflammation of the kidney organs. Pravastatin has pleiotropic abilities that can be anti-inflammatory, antioxidant and anti-apoptotic in preeclampsia conditions

Purpose of the study: Knowing the effect of pravastatin administration on kidney repair in preeclampsia rats.

Subjects and Methods: Post-test group only experimental study using kidney tissue model of preeclampsia rats Rattus norvegicus (exposed to L-NAME) and given pravastatin at various doses (2 mg, 4 mg, and 8 mg).

Outcomes: The results of One Way Anova test on glomerular area, arteriole diameter and number of inflammatory cells (lymphocytes and monocytes) in five study groups had a p-value of < 0.05 (0.000, 0.000, 0.000). The correlation test of pravastatin dose to glomerular area variables with pravastatin dose was (p-value 0.000; r = -0.819), arteriole diameter (p-value 0.000; r = 0.904), the number of inflammatory cells was (p-value 0.001; r = -687) and epithelial cell necrosis proximal tubule (p-value 0.000, r= -0.668).

Conclusion: Pravastatin can increase arteriole diameter, decrease glomerular area and decrease the number of inflammatory cells (lymphocytes and monocytes) and decrease the number of epithelial cells that undergo necrosis in kidney tissue model of preeclampsia wistar rats (Rattus norvegicus).

INTRODUCTION

Preeclampsia is high blood pressure (systolic ≥ 140 mmHg and diastolic ≥ 90 mmHg) accompanied by proteinuria or without proteinuria, persistent headaches, visual disturbances and even seizures (eclampsia) in pregnant women at > 20 weeks gestation (1). Preeclampsia is the cause of death in 70,000 mothers and 500,000 infant deaths worldwide (2). In 29 countries, preeclampsia also accounts for nearly 30% of all maternal deaths (20 per 100,000), and mortality rates are 0.8% in affected women (3).

According to data from the Indonesian Health Profile (2020), maternal mortality cases increased by 406 cases from 4221 cases in 2019 to 4.627 cases in 2020. The contributors to Maternal Mortality Rate (MMR) in Indonesia are: bleeding (1,330 cases or 28.78%), hypertension in pregnancy (preeclampsia) i.e. 1,110 cases (24.02%) and circulatory system disorders (230 cases or 5%) (4)

The cause of preeclampsia is not known with certainty however, Preeclampsia is understood as a disease that begins with an abnormal placenta and maternal syndrome. Preeclampsia is mediated by abnormal placenta and superficial cytotrophoblast invasion in the uterus resulting in incomplete remodeling of the spiral arteries resulting in placental ischemia, angiogenic imbalance, endothelial dysfunction, oxidative stress and systemic inflammation (5).

Preeclampsia causes stunted fetal growth, pulmonary edema, intracranial hemorrhage, bleeding complications, eclampsia in the mother, increased risk of kidney damage later in life (3). The kidney organ is a filtration site for metabolic substances, however, in the state of preeclampsia there are structural changes in the glomerulus, renal tubules and arterioles and occurs in kidney function (6). In pregnant conditions the blood flow rate will increase but in preeclampsia there is a decrease of 30-40% Glomerular filtration rate (GFR), creatinine and urea rates are caused by vasoconstriction of blood vessels or narrowing of the diameter of arterioles in the kidneys causing blood pressure to increase and ischemia and hypoxia occur (7).

The condition ischemia causes The body will expel *Soluble Factor* and *Debris* into the blood circulation and causes increased oxidative stress in preeclampsia leads to increased production of Reactive Oxygen Species (ROS) followed by endothelial vasoconstrictors causing vasoconstriction, congestion, hypoperfusion, and the expression of adhesion molecules affects glomerulus and proximal tubules in the kidneys (8).

The glomerulus undergoes enlargement, swelling of endothelial cells and loss of glomerular endothelial fenestra (glomerular capilari endotheliosis) resulting in the accumulation of creatinine and urea in body fluids and increasing the concentration of creatinine in plasma (9). The most pronounced picture in glomerular lesions, a slight increase in volume and a decrease in the diameter of the capillary lumen. An increase in glomerular volume correlates with the severity of the patient's clinical condition. Glomerular changes occur due to endothelial swelling, endothelial fenestra vacuolization, and encroachment in the capillary lumen, a combination of these changes called endotheliosis (10).

The proximal tubule will also be seriously affected by preeclampsia because the proximal tubule composed of many mitochondria requires high amounts of oxygen to produce ATP for cell survival (11). If hypoxia occurs, oxidative stress will occur causing increased production*Reactive Oxygen Species* (ROS) and expression of adhesion molecules (12). The expression of adhesion molecules and cytokines produced by the tubule epithelium will initiate the infiltration of lymphocyte cells and monocytes, resulting in microcirculation obstruction so that inflammatory cells migrate to the interstitial tissue of the proximal tubule (13). The release of cytokines and ROS by such lymphocytes and monocytes causes biochemical changes and provokes pathological conditions by damaging the epithelium of the tubules causing necrosis of the epithelial cells of the proximal tubules (14). If it lasts for a long time, it will damage the kidney organ (8).

Preeclampsia is difficult to treat because it involves many mediators and affects several organs so preventive steps and appropriate treatment are needed. Pravastatin is a new therapeutic candidate as a preventive therapy by inhibiting the enzyme HMG-CoA reductase so as to reduce the amount of cholesterol produced by the body (15).

Pravastatin has been shown to improve the pathophysiological pathways underlying the development of preeclampsia. Pravastatin also has anti-inflammatory effects and has potential in protecting organs including the kidneys from inflammation-induced damage (16). In addition, pravastatin can also improve endothelial function, reduce oxidation, and inhibit platelet aggregation, which can help protect the kidneys from further damage (15) (17)

The effect of pravastatin has been tested in animal models of preeclampsia by Ahmed *et al.,* A 2010 report that 20 mg/kg/body weight/day of pravastatin treatment prevented the occurrence of preeclampsia in immunologically mediated mouse models, which spontaneously developed pathological changes associated with preeclampsia. Results of Rahardjo's research *et al.,* (2022) proved that pravastatin can increase the expression of VEGF, eNOS, PECAM-1 and decrease the expression of IL-10, TNF-α, and sFlt-1 in the placenta of mouse models preeclampsia with optimal dosage 2mg/kg/BB/day. The reason for the use of pravastatin as a preventive alternative to preeclampsia is based on experimental animal studies, which showed that pravastatin (3-hydroxy-3-methylglutaryl Coenzyme-A-reductase inhibitor) has a possible protective role of uteroplacental and vascular endothelial cells. Pravastatin has an effect *pleiotropic* which is protective against preeclampsia such as endothelial protection, antioxidant properties, anti-inflammatory effects, effects antithrombotic, and the most relevant pro-angiogenic effects (16)(18)(17). Pravastatin as a new candidate to prevent preeclampsia and as protection of kidney organs from the effects of preeclampsia by assessing glomerular area, renal arteriole diameter andn number of inflammatory cells (Lymphocytes and monocytes) at mouse kidney model of preeclampsia.

SUBJECT AND METHOD

This research was conducted at the Bioscience Laboratory of the Faculty of Medicine, Universitas Brawijaya Malang. we used experimental animals of the wistar strain rat (*Rattus norvegicus*). This study used a *post test only cotrol group design.* This study was conducted in vivo with experimental animals of Wistar strain rats divided into 5 groups consisting of negative control (untreated mice), positive control (preeclampsia model mice), and 3 treatment groups (P1, P2 and P3). The parameters studied are arteriole diameter, glomerular area, and number of inflammatory cells. This research group is divided into five groups including:

K (-) : Negative control (without any intervention).
K (+) : Positive Control (L-Name 125mg/kg/BB) or preeclampsia model
P1 : L-NAME 125mg/kg /BB + pravastatin 2mg/kg/BB
P2 : L-NAME 125mg/kg /body weight + pravastatin 4mg/kg/body weight
P3 : L-NAME 125mg/kg /BB + pravastatin 8mg/kg/BB

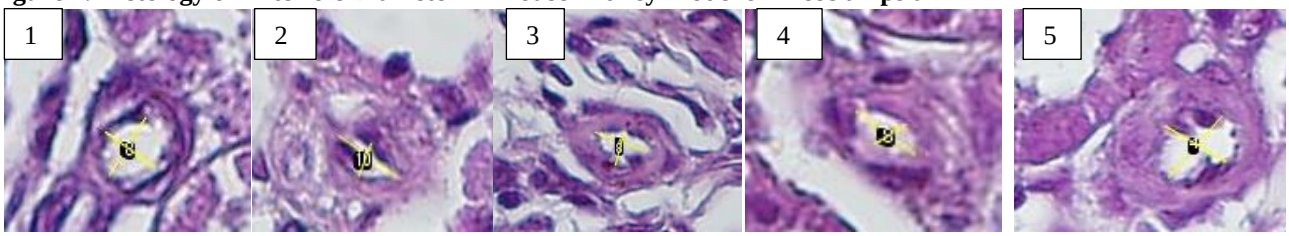
This study began cutting kidney tissue samples with a thickness of 3-5 mm. The cut tissue is put into a tissue cassette label and then closed. then dehydration, purification, embedding, cutting with microtomes with a thickness of 2-4 µm until the slide is ready to be stained Hematoxcylin Eosin is then analyzed with an Olympus Bx 53 Binocular microscope with a bar scale of 5 µm, examination using image J (Fijian version) and perform SPSS 23 data analysis. This study used five data analysis steps: (1) *Shapiro-Wilk data normality* test, (2) Statistical Homogeneity test, *Levene* test , (3) One Way ANOVA test, (4) Post-Hoc Tukey HSD test test, (5) *Pearson correlation test*. This research has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya.

RESULT

Effect of Pravastatin on Arteriole Diameter

The results of the study used HE staining with Olympus BX 53 Binocular microscope with 400x magnification:

Figure 1. Histology of Arteriole Diameter in Mouse Kidney Model of Preeclampsia



Remarks : (1-5) difference in arteriole diameter in kidney tissue, magnification 400x,; (1) Negative Control K(-) ; (2) Positive Control K (+) i.e. kidney of preeclampsia model bunting rats; (3) P1, namely the kidneys of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB (4) K2 namely the kidneys of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB; and (5) K3, namely the kidneys of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB,

Table 1. Effect of Pravastatin on Arteriole Diameter

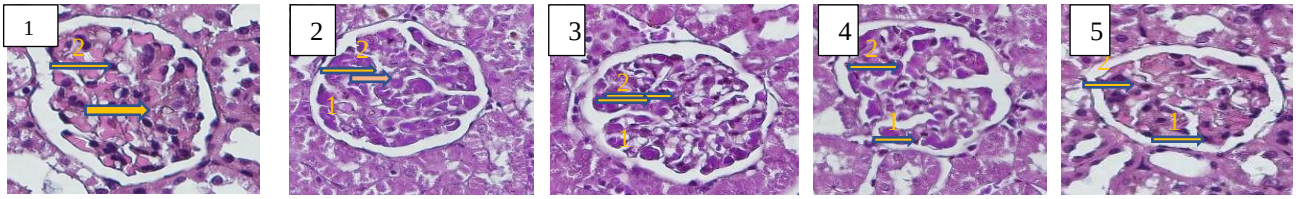
Research Group	N	Average ± SD (um)	*p value* (ANOVA)
Negative Control	5	23.3 ± 2.86s	
Positive control	5	11.31 ± 0.57a	
Treatment 1	5	15.22 ± 0.78b	**0,000<α**
Treatment 2	5	17.51 ± 0.67bc	
Treatment 3	5	18.86 ± 0.48C	

Note: *A p-value of < 0.05 means there is a significant difference and a p-value of 0.05 means there is no significant difference. The same letter means there is no significant difference (p<0.05).*

Effects of Pravastatin on Glomerular Area

Figure 2. Extensive Glomerular Histology in Mouse Kidney Model of Preeclampsia

1



Remarks : (1-5), Glomerular area of mouse kidney with HE staining at 400x magnification of glomerular area measured by ImageJ. (1) : Negative control arrow 1 : normal podocyte cells, arrow 2 : normal mesangial cells; (2) : Positive Control, shown arrow 1 : poscite cells undergo vacuolization and mesangial matrix proliferate (3) : Treatment 1 (Pravastati dose 10 mg) shown with arrow 1 : posocyte cells still vacuolize , arrow 2 : mesangial matrix still undergoing proliferation; (4) : Treatment 2 (Pravastatin dose 20 mg) shown arrow 1: vacuolized podocyte cells have improved, arrow 2: mesangil matrix that has proliferated has improved slightly; (5): Treatment 3 (Pravastatin dose 40 mg) shown with arrow 1: poodcyte cells are starting to look normal and mesangial cells have seen improvement.

Table 2. Effect of pravastatin on Glomerular Area

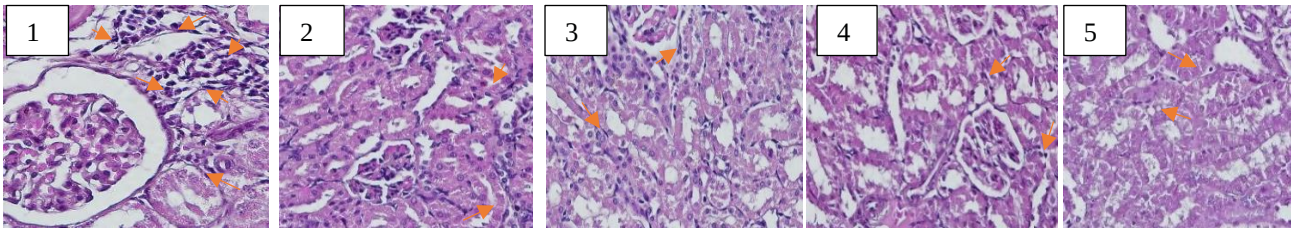
Research Group	N	Average ± SD (um)	*p value* (ANOVA)
Negative Control	5	322.05 ± 15.25a	
Positive control	5	394.05 ± 5.96c	
Treatment 1	5	356.04 ± 18.93b	**0,000<α**
Treatment 2	5	348.01 ± 9.61ab	
Treatment 3	5	327.01 ± 9.72a	

Note: *A p-value of < 0.05 means there is a significant difference and a p-value of 0.05 means there is no significant difference. The same letter means there is no significant difference (p<0.05).*

Effects of Pravastatin on the number of inflammatory cells

The results of the study used *Hematoxcylin Eosin* staining with Olympus BX 53 Binocular microscope 400x magnification with 5µm scala bar:

Figure 3. Histology of inflammatory cell counts (lymphocytes and monocytes) in mouse kidneys model of preeclampsia



Remarks : (1-5) differences in the number of inflammatory cells (lymphocytes and monocytes) in kidney tissue with a magnification of 400x and a bar scale of 5 µm; (1) Negative Control K(-) ; (2) Positive Control K (+) i.e. kidney of preeclampsia model bunting rats; (3) P1, namely the kidney of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB (4) K2 namely the kidney of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB; and (5) K3, namely the kidney of pregnant mice model of preeclampsia + pravastatin 2 mg / KgBB, inflammatory cells are characterized by the appearance of dark purple lymphocyte cells with dense chromatin while monocyte cells with cytoplasm envelop the nucleus and have a large grayish nucleus. Inflammatory cells are shown with orange arrows.

Table 3. Effect of Pravastatin on the Number of Inflammatory Cells

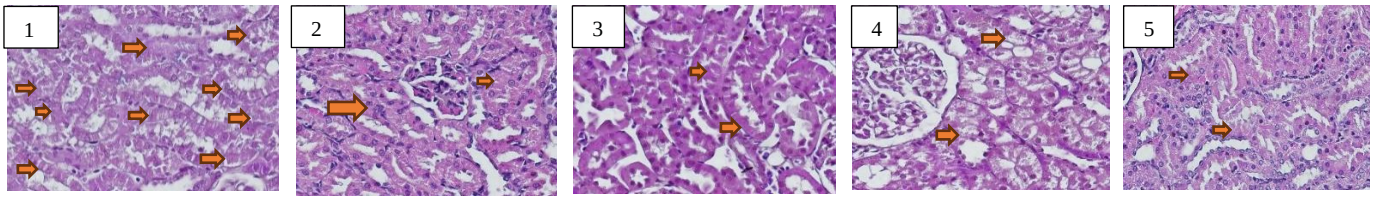
Test with *One Way Anova* with the average result of the number of inflammatory cells in 5 treatment groups with a p-value of < 0.05 (0.000)

Research Group	(N)	Average ± SD (%)	One Way Test (ANOVA)
Negative Control	5	16.92 ± 4.80 [a]	
Positive control	5	32.70 ± 4.77 [b]	
Treatment 1	5	21.26 ± 4.75 [a]	0,000 <α
Treatment 2	5	6.00pm ± 1.27 [a]	
Treatment 3	5	16.94 ± 6.32 [a]	

Description: In the mean ± SD, if different letters mean there is a real difference (p-value < 0.05) and if they contain the same letters means there is no significant difference (p-value > 0.05). K (-) is the liver of a normal pregnant mouse. K (+) is the kidney of preeclampsia mice that received an injection of L NAMA 125mg/kgBB. P1 is the kidney of preeclampsia rats + pravastatin 2 mg / kg body weight . P2 is the kidney of preeclampsia rats + pravastatin 4 mg/ kgBB . P3 is rat kidney preeclampsia + pravastatin 8 mg/ KgBB.

Effects of pravastatin against proximal tubular epithelial cell necrosis

Figure 4. Histology of proximal tubular epithelial cell necrosis in mouse kidney model of preeclampsia



Remarks : (1-5) Differences in the number of proximal tubular epithelial cells that undergo necrosis in rat kidney tissue with HE staining at 400x magnification with a bar scale of 5 μm; (1) Negative Control K(-) ; (2) Positive Control K (+) i.e. kidney of preeclampsia model bunting rats; (3) P1, namely the kidney of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB (4) K2 namely the kidney of pregnant mice model of preeclampsia + pravastatin 2 mg / KgBB; and (5) K3, namely the kidneys of pregnant rats model of preeclampsia + pravastatin 2 mg / KgBB, Proximal tubular epithelial cells undergo necrosis indicated by the disappearance of the cell nucleus, unclear cell membrane structure and vacuole cytoplasm; The orange arrow ( ) is an epithelial cell that undergoes necrosis

Table 4. Effect of pravastatin on Proximal tubular epithelial cell necrosis

Research Group	N	Average ± SD	*p value* (ANOVA)
Negative Control	5	13.54± 1.39 [A]	
Positive control	5	74.08± 6.43 [b]	
Treatment 1	5	7.10pm ± 2.52 [a]	0,000 < α
Treatment 2	5	17.10± 3.92 [A]	
Treatment 3	5	19.22± 3.79 [A]	

Note: *A p-value < 0.05 means there is a significant difference and a p-value > 0.05 means there is no significant difference. The same letter means there is no significant difference (p<0.05).*

Table 5. Pearson Correlation Test

Variable	Correlation coefficient (r)	*P-Value*
Pravastatin dosage against extensive glomerulus	-0.819	0.000
Pravastatin dosage to arteriole diameter	0.904	0.000
Pravastatin dosage against the number of inflammatory cells	-0.687	0.001

Pravastatin dosage against proximal tubular epithelial cell necrosis	-0.668	0.001

Note: The significance value is 5% or 0.05. If the correlation result is negative it means inversely proportional, if the correlation result is positive then it is directly proportional. If the significance value > 0.05, it means that there is no relationship between the marker and the dose of pravastatin.

The pearson *correlation test* shows that the correlation between glomerular area with pravastatin dose is -0.819 with a p-value of 0.000 with a negative correlation that the higher the dose of pravastatin, the glomerular area decreases, the correlation in arteriole diameter with pravastatin dose is 0.904 with a p-value of 0.000 which shows a positive correlation that the higher the dose of pravastatin given, the more increased the diameter of the arteriole, the correlation of the dose of pravastatin with inflammatory cells is r = -0.678 and *P-value* 0.001 with the direction of negative correlation between that the higher the dose of pravastatin, the number of inflammatory cells (lymphocytes and monocytes) decreases and the correlation of epithelial cell necrosis with the dose of pravastatin which is r = -0.668 with a p- value of 0.001 with a negative correlation direction means that the higher the dose of pravastatin, the number of epithelial cells that experience necrosis in the proximal tubule is more Decreased.

DISCUSSION

Preeclampsia causes injury to the kidneys

In preeclampsia there is kidney organ dysfunction caused by increased antiangiogenesis factors (s-Flt1 and sEng) and inhibiting the production of pro angiogenesis (VEGF and PlGF) so that vascular vasoconstriction occurs resulting in blood vessels having a small diameter while in pregnant conditions the effective plasma flow rate increases up to 80%, and GFR increases by 40-60%. if GFR decreases, it will cause damage to glomerular endothelial cells, causing an increase in hypoxia-induced stress mediators resulting in oxidative stress and inflammatory response (19) (20)

Increased oxidative stress in preeclampsia leads to increased production *Reactive Oxygen Species* (ROS) followed by endothelial vasoconstrictors will cause vasoconstriction, congestion, hypoperfusion, and expression of adhesion molecules (21). The expression of adhesion molecules and cytokines produced by tubular epithelium will initiate the infiltration of inflammatory cells, namely leukocytes and monocytes, and microcirculation obstruction occurs so that inflammatory cells migrate to the tissues (22). The release of cytokines and ROS by such leukocytes and monocytes causes biochemical changes and provokes pathological conditions by damaging the epithelium of the tubules. If it lasts for a long time, there will be acute tubular epithelial cell necrosis and will damage the kidney organ (23).

Pravastatin is able to enlarge the diameter of arterioles

In preeclampsia conditions ischemic kidney injury occurs characterized by striking hemodynamic changes that cause GFR to decrease (24) One of the hemodynamic changes that can be seen is vasoconstriction where blood vessels are narrowed so that the blood supply to the kidneys, especially the glomerulus, decreases due to narrowing in the diameter of blood vessels (25). The diameter of the renal arterioles plays an important role in regulating vascular resistance, glomerular filtration rate and maintaining optimal kidney function (7).

In addition, it is believed that blood vessels fail to reshape and drain enough blood to the placenta, resulting in chronic ischemia. As a result, the placenta produces factors such as sFlt-1 which is associated with maternal syndrome. This results in endothelial dysfunction and vasoconstriction, renal perfusion failure and eventually hypertension (26)**.** In pregnant women who experience preeclampsia caused by vasoconstriction of renal blood vessels can cause a decrease in GFR of up to 32% followed by a decrease in renal plasma flow of up to 24% (27)**.**

The mean arteriole diameter in this study was higher in the group of mice receiving pravastatin therapy compared to the group of preeclampsia model mice with significant differences in the P1, P2 and P3 groups. The diameter of the arterioles in the group given pravstatin in the P2 and P3 groups at doses of 4 mg / kg and 8 mg / kg proved to be greater than the diameter of the arterioles of the positive group where in the condition of preeclampsia the diameter of the renal arterioles narrowed. This suggests that pravastatin may exert a protective effect against proangiogenic factors in preeclampsia kidneys.

Statins can improve the function of the kidneys. Statin therapy can amplify the effect of other endothelium-related drugs, improve blood flow, reduce the propagation of surface adhesion molecules. This is due to the ability of statins to regulate the expression of endothelial nitric oxide synthase (eNOS) which can increase the production of NO and help blood vessel reaction. In addition, statins have also been found to promote the proliferation, migration and survival of circulating endothelial procinietor cells; this is important in angiogenensis and endothelial restoration after injury (28) (17).

Pravastatin is able to decrease the area of the glomerulus

In general, preeclampsia interferes with renal perfusion at both renal and renal levels.(29) Changes in the renal part, where the glomerulus is enlarged accompanied by swelling of endothelial cells and loss of glomerular endothelial fenestra (glomerulur capillary

endotheliosis) this results in the accumulation of creatini and urea in body fluids and increases the concentration of creatini and urea in plasma (9). Damage to the renal endothelium called glomerular endotheliosis is the dilation and hardening of the glomeruli wall with little blood flow which causes narrowing and even blockage of the capillary lumen which will lead to swelling of podocyte cells, mesangial cells and blockage or occlusion in the capillary lumen (30)**.** Glomerular changes occur due to endothelial swelling, endothelial fenestra vacuolization and encroachment on the capillary lumen, these changes are called endotheliosis which can result in capillary lumen occlusion and placental ischemia (10).

The mean glomerular area in this study was lower in the group of rats who received pravastatin administration compared to the group of mice in the preeclampsia model group with significant differences in the P1, P2 and P3 groups. The area of the glomerulus given pravastatin at a dose of 8 mg / kg at P3 proves to be reduced which in the condition of preeclampsia the glomerular area has swelling due to endotheliosis. This shows that pravastatin can provide a protective effect against proangiogenic factors so that angiogenic balance can return to normal. The results of this study support several previous studies related to the protective effects of pravastatin on the kidneys.

Pravastatin as an HMG-CoA reductase inhibitor that works as an inhibitor of the activity of HMG-CoA reductase which can affect Heme Oxygenase-1 (HMOX-1) in CO production and eNOS activation so that NO production increases. Increased NO will cause a decrease in ROS which will also reduce oxidative stress to inflammation. In addition, increased NO production can restore angiogenic balance so that it can repair endothelial damage and provide protection to the endothelium (17).

Pravastatin is able to reduce the number of inflammatory cells (lymphocytes and monocytes)

Inflammation can provide feedback to increase ROS formation or stimulate cytokine and growth factor production (31). Cytokines are polypeptides that regulate many important biological processes, acting as mediators of inflammation and immune response. Cytokines are closely associated with the repair of damaged tissue and are potential biomarkers of nephrotoxicity because they are involved in damage and repair (32). In addition, ROS also modulates the production of helper T cells (Th) 2 and IL-4 production and activation of Th 2 cells which will produce pro-inflammatory cytokines such as IL-4 and IL-5, activation of macrophages, mast cells and eosinophil response will later cause inflammatory cell infiltration in the interstitial (33).

In accordance with the theory above, the results of this study also had a higher average number of inflammatory cells in the positive group or preeclampsia model mice given L-Name (32.70) compared to the normal group (16.92) and P1 group (21.26), P2 (18.00) and P3 (16.94) with the most significant difference in the P3 group. This means that pravastatin is able to provide a protective effect against proangiogenic factors and as an anti-inflammatory in preeclampsia kidneys.

It has been reported that some (though not all) statins selectively inhibit an important inflammatory cell adhesion protein, the integrin aLb2 (also referred to as antigen 1 associated lymphocyte function, or integrin LFA-1). Statin therapy has also been found to affect immune cell signaling. Interferon gamma plays an important role in the immune response by stimulating immune cells to express the major histocompatibility complex class II (MHC-II) protein, which in turn activates T lymphocytes. There is evidence that statins directly inhibit the induction of MHC-II expression-mediated interferon gamma leading to decreased T cell activation. Through inhibition of T cell activation and expression of adhesion molecules, Statins decrease the presence of inflammatory cytokine-releasing immune cells (monocytes, macrophages, lymphocytes) in the endothelium (34) (35) (36) (17) (37)

The results of other studies also prove that pravastatin is able to improve inflammatory states by increasing proangiogenic so that there is a decrease in the number of inflammatory cells (lymphocytes and monocytes). Statins have anti-inflammatory properties and have been shown to lower hs-CRP even in patients with normal cholesterol levels. Pravastatin regulates the production of anti-inflammatory cytokines Th2 and decreases the production of proinflammatory cytokines Th1. The immunomodulatory and anti-inflammatory effects on statins are pleiotropic actions as the formation of free oxygen radicals and smooth muscle cell proliferation, making statins very promising for the prevention and treatment of preeclampsia (37) (18)

In line with the results of this study that pravastatin has the ability to suppress the action of Hydroxymethylglutaryl-CoA, also known as HMG-CoA. This induces Hmox-1 to catabolism carbon monoxide and activates eNOS for the formation of NO, both of which work as vasodilators in the process of maintaining angiogenic balance. In preeclampsia, administration of pravastatin can stimulate the release of VEGF and inhibit the synthesis of sFlt-1 thereby reversing angiogenic imbalance thereby preventing preeclampsia and reducing the number of inflammatory cells (lymphocytes and monocytes) (18) (17)

Pravastatin is able to reduce the number of epithelial cells that undergo necrosis in the proximal tubule

The result of this study is that by being given pravastatin, the number of epithelial cells experiencing necrosis will decrease. In accordance with the average results, the number of proximal tubular epithelial cells that experienced necrosis in this study was highest in the positive group or the preeclampsia model mouse group (mice given L-Name) with an average (74.08) compared to the normal mouse group (13.54), P1 (19.10), P2

(17.10) and P3 (19.22) with significant differences in the P2, P1, and P3 groups. This means that pravastatin is able to provide protection / protection to the integrity of the proximal tubular epithelial cell membrane in preeclampsia kidneys with a certain dose.

In line with the results of this study that statins have an inhibitory effect on the formation of free radicals. In addition, statins also have beneficial effects on monocyte recruitment, mesangial cell proliferation, endothelial function, renal hemodynamics, and mesangial matrix accumulation as well as anti-inflammatory and immunomodulatory activities. In theory, each of these mechanisms might mediate the assumption of statin's renoprotective properties (17). Where it is known that the pathophysiology of tubular necrosis begins with tubule injury due to ischemia so that ATP depletion occurs rapidly due to oxygen depletion causing formic acid to inhibit work *cytochrome C oxidase* in mitochondria and mitochondria cannot produce ATP so cell hypoxia occurs (38)

ATP depletion causes disruption of the cytoskeleton of the proximal tubular epithelium and loss of microvilli accompanied by the transfer of integrin locations from the basal surface to the apical surface causing epithelial cells to detach and leak because the wall is not lined with epithelium causing filtrate to leak and then re-enter the circulation thus activating *Protease* which causes oxidative injury to the tubule epithelium and capillary endothelium due to the formation of Reactive Oxygen Species (ROS) along with endothelin vasoconstrictors will cause vasoconstriction, congestion, hypoperfusion, and expression of adhesion molecules (39)(38). The expression of adhesion molecules and cytokines produced by the tubule epithelium will initiate leukocyte infiltration, resulting in microcirculation obstruction. The release of cytokines and ROS by these leukocytes can damage the tubular epithelium resulting in acute tubular necrosis (40)

Based on the theory above, the use of statin therapy can be very helpful because in accordance with its function, namely as endothel repair, block the production of free radicals and will help the repair process after injury (15). The results of previous studies related to the protection of pravastatin in the kidneys by regulating endothelial expression *nitric oxide synthase* (eNOS) which increases NO production and promotes relaxation of blood vessels. Statins have also been found to restore eNOS function in pathological conditions and increase the expression of tissue-type plasminogen activators and decrease the expression of potent vasoconstrictors ET-1. If eNOS functions properly, it will activate NO so that it will produce proangiogenic VEGF and PIGF functions as vasodilation, maintaining oxygen supply into the body and preventing hypoxia, vasoconstriction and oxidative strees so that if hypoxia, vasoconstriction and oxidative strees do not occur, cells will not experience cell damage or death, especially in proximal tubular epithelial cells which are very susceptible to hypoxic conditions due to proximal tubules composed of many mitochondria that require large amounts of oxygen (produce ATP) for the survival of epithelial cells. In addition, statins have been found to promote the proliferation, migration, and survival of circulating endothelial progenitor cells, which are important for angiogenesis and endothelial restoration after injury ((16) (17)(15)

CONCLUSION

Administration of pravastatin can increase arteriole diameter, decrease glomerular area and reduce the number of inflammatory cells (lymphocytes and monocytes) in kidney tissue model of preeclampsia wistar rats (Rattus norvegicus). This effect correlates with an increase in the dose of pravastatin. The nature and mechanism of action of pravastatin make it a very promising candidate for the prevention of preeclampsia. The use of pravastatin for the prevention of preeclampsia in clinical circumstances requires further investigation.

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Authors' contributions
All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.
Declaration of Conflicts of Interests
Authors declare that they have no conflict of interest.
Data Availability Statement
The database generated and /or analysed during the current study are not publicly available due to privacy, but are available from the corresponding author on reasonable request.
Declarations
Author(s) declare that all works are original and this manuscript

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