Preface: The 4th International Conference on Life Science and Technology (ICoLiST)

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Preface: The 4th International Conference on Life Science and Technology (ICoLiST)

The 4th International Conference on Life Science and Technology (ICoLiST) brings together a wide range of researchers, academicians, and industry experts from research areas of life science and technology, from the theoretical perspectives to the advanced applications. This annual conference was held virtually via Zoom on 31st August 2021 by Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Indonesia, Institut Teknologi Sepuluh Nopember, Universiti Teknologi Malaysia, Universitas Gadjah Mada, Tsing-Hua University, Institut Pertanian Bogor, FH Aachen University of Applied Sciences, IAIFI Cabang Malang, The University of Newcastle, Universitas Islam Negeri Maulana Malik Ibrahim, Hiroshima University, Universitas Muhammadiyah Malang, Wageningen University and Research, Universitas Islam Malang, Universitas Brawijaya, Sekolah Tinggi Ilmu Kesehatan Hafshawaty Zainul Hasan, Universitas Trunojoyo Madura, Universitas Khairun, Akademi Keperawatan Dharma Husada, UIN Sulthan Thaha Saifudin Jambi, Gamma Scientific Biolab, Nikon, and Genecraft Labs.

This is the second year we held the conference virtually due to the pandemic. Since the current global health crisis showed us the crucial contribution of science, technology and innovation to address global challenges brought about by COVID-19, we facilitated this scientific platform to enrich us with new inspirations, innovations and solution to deal with the challenges, particularly, with the contributions of the leading research in the field of life sciences and technology.

The conference brought more than 800 participant delegates from nine countries (Indonesia, Australia, Germany, Netherlands, Malaysia, Taiwan, Japan, Thailand, dan Philippine) including biologist, physicist, chemist, biotechnologist, doctors and environmental engineers whose interest pertain to the current research issues on life science and technology. Oral presentation and poster addressed 13 main topics incorporating botany, zoology, physiology, microbiology, biochemistry, biophysics, biotechnology, biomedical science, bioradiation, bioconservation, bioinformatics, biomaterial science, nanotechnology, and environmental engineering.

Ultimately, we would like to express our gratitude to all the keynote and invited speakers, reviewers, participants, delegates, and all committee members for the commitment to contributing and support this great event.

Chief Editors

Prof. Dr. Ahmad Taufiq, M.Si.

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Entin Srihadi Yanti, Rasyidah Rasyidah, Bambang Rahardjo, et al.

Transforming Growth Factor-B and Interleukin-10 Levels on Preeclampsia Rat Model Treated Pravastatin

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Abstract. Preeclampsia is a clinical syndrome characterized by proteinuria and hypertension after 20 weeks of pregnancy. Pathophysiology of preeclampsia is affected by an imbalance of systemic angiogenic factors and immunological factors. TGF-β can represent a proangiogenic factor in preeclampsia and IL-10 is one of the anti-inflammatory cytokines found to be low in preeclampsia conditions. Pravastatin is an antihyperlipidemic drug candidate for a potential therapy for preeclampsia. This study aims to determine the effect of pravastatin on TGF-β and IL-10 serum levels in the preeclampsia rat model. It used five groups consisting of negative control (normal pregnant rats) group, positive control (preeclampsia rat model induced L-NAME 125 mg/Kg BW) group, preeclampsia rat model groups given pravastatin in 3 different doses (2 mg/day, 4 mg/day, and 8 mg/day). On days 12, 15, and 19, the blood pressure of pregnant rats was measured. The serum was collected after the rats were sacrificed in order to determine TGF-β and IL-10 levels. The result showed an increase in TGF-β serum level (p=0.003) and IL-10 serum level (p=0.001). Therefore, pravastatin can increase TGF-β and IL-10 levels significantly in preeclampsia rat models.

INTRODUCTION

Preeclampsia is characterized by the onset of hypertension, proteinuria, hematological complications, and uteroplacental disorders [1],[2]. The definite primary etiology responsible for the onset of preeclampsia remains unknown, but "two-stage theory" can explain the possible pathophysiology of preeclampsia [3]. The first stage is abnormal placentation that can cause oxidative stress, while the second stage is maternal vascular endothelial dysfunction which is come from a combination of several processes, including imbalance of angiogenic factors, inflammatory response [3],[4]. The imbalance of angiogenic factors is characterized by excessive antiangiogenic factors in the maternal circulation and decreased levels of proangiogenic factors in the blood circulation, including TGF-β [5],[6],[7]. The inflammatory response can be shown by IL-10, an anti-inflammatory cytokine that is found to be low in preeclampsia conditions [8],[9].

Prevention and treatment of preeclampsia are still limited [3],[10]. Statins have several pleiotropic effects by increasing the expression of HMOX-1, thereby inhibiting HMG-CoA [11],[12]. As a proangiogenic, statins improve the regeneration of vascular endothelium by increasing the viability of endothelial progenitor cells and are promoted at the sites of ischemia. As an anti-inflammatory, statins have been shown to quench the inflammatory response and reduce endothelial inflammation as well as stabilize plaque. Pravastatin, specifically, has been reported in various preclinical and clinical studies to reverse the pregnancy-specific angiogenic imbalance associated with preeclampsia, restore global endothelial health, and prevent oxidative and inflammatory injury [13].

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The effect of pravastatin on pregnancy is significant to study, but it is too difficult if the pathomechanism and causal relationship are studied directly in humans [2],[14],[15]. Therefore, the animal model of preeclampsia was used to test the effect of pravastatin on TGF-β and IL-10 levels in preeclampsia Induction of preeclampsia using Nitro *L-Arginine Methyl Ester* (L-NAME).

EXPERIMENTAL DETAILS

This study used a true experimental design, with a post-test only control group design. The samples were 20 pregnant rats divided into 5 groups so that each group consisted of 4 samples. The negative control group consisted of normal pregnant rats, the positive control group consisted of preeclampsia pregnant rats, while treatment groups 1,2, and 3 were preeclampsia pregnant rats given pravastatin in three different doses (2 mg/day, 4 mg/day, and 8 mg/day). The first day of pregnancy was determined at 1×24 hours after matting. Termination was carried out on the 19th day of pregnancy. The samples taken were serum TGF-β and IL-10. This research was implemented in the Bioscience Laboratory of Brawijaya University and Physiology Laboratory of the Faculty of Medicine, Brawijaya University Malang. The procedure on experimental animal models had previously received ethical approval from the Ethics Committee of the Faculty of Medicine, Brawijaya University Malang, Indonesia (Code of Ethics: 13/EC/KEPK/01/2021).

Animal models of preeclampsia could be created by injecting L-NAME (C7H=N5O4.HCl) 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 determination of animal models of preeclampsia was determined based on increased Blood Pressure (> 140/90) from the results of blood pressure measurements. 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.

Blood pressure was measured 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 13th, 15th, and 19th days of pregnancy, while urine protein was measured using the urinalysis reagent test strips method on the 13th, 15th, and 19th days of pregnancy. TGF-β levels were measured by the ELISA method with no. Catalog E-EL-R0084 and IL-10 levels were measured using the ELISA method with No. E-EL-R0016 Catalog produced by Elabscience. Data analysis was completed with SPSS 25.00. The statistical test used was the One Way Annova test as a different test followed by the LSD test. The effect of dose on serum levels of TGF-β and IL-10 was determined by using a correlation test.

RESULTS AND DISCUSSION

Since the 13th day of gestation, L-NAME 125 mg/kg/day was injected intraperitoneally and showed an increase in the average systolic blood pressure (Figure 1) and urine protein (Figure 2) in pregnant Wistar rats (Rattus norvegicus).

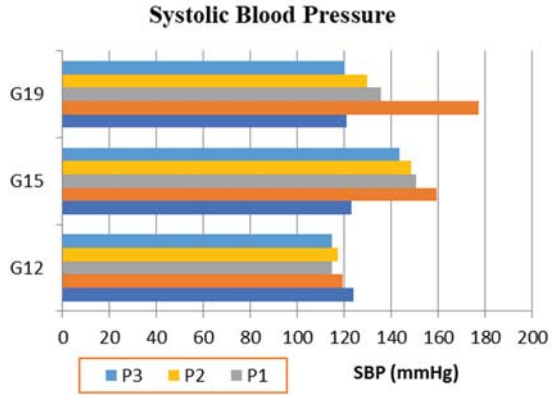

FIGURE 1. Systolic Blood Pressure Measurements in Preeclampsia Rats Pregnant Model Given Different Doses of Pravastatin. The mean systolic blood pressure of pregnant rats model of preeclampsia was measured at the 12th, 15th, and 19th gestation. K(-) is a group of normal pregnant rats, K(+) is a pregnant rat model of preeclampsia, P1, P2, and P3 are rats of a preeclampsia model given pravastatin with doses of 2 mg/day, 4 mg/day and 8 mg/day.

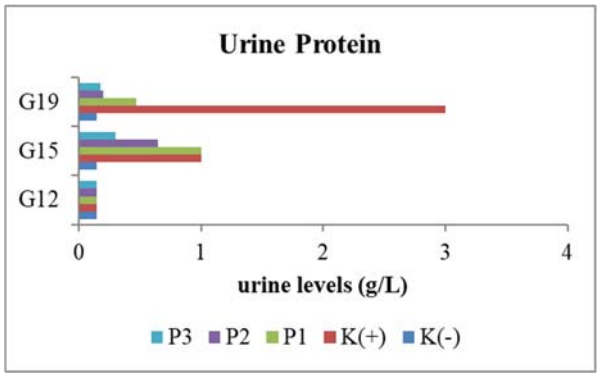

FIGURE 2. Results of Urine Protein Measurement in Preeclampsia Rats Pregnant Model Given Different Doses of Pravastatin. The mean urine protein of pregnant rats model preeclampsia was measured at the 12th, 15th, and 19th gestation. K(-) is a group of normal pregnant rats, K(+) is a pregnant rat model of preeclampsia, P1, P2, and P3 are rats of a preeclampsia model given pravastatin with doses of 2 mg/day, 4 mg/day and 8 mg/day.

The proteinuria was measured at 12, 15, and 19 gestations. L-NAME injection of 125 mg/kg BW/day can increase systolic blood pressure and urine protein in Preeclampsia Rats Pregnant Model. The injection of L-NAME 125 mg/kg/day in pregnant rats caused by a pathological set resembling preeclampsia through the eNOS uncoupling process. Clinical signs of intraperitoneal L-NAME injection are also found to increase systolic blood pressure and urine protein >+1 [16],[17]. There is a decrease in TGF-β production in preeclamptic rat models with L-NAME injection. The study results signify that the level of TGF-β in preeclampsia rats is lower than the group of normal pregnant rats. The results of this study are in line with research conducted by Yusrawati *et al.,* which states that TGF-β levels are significantly lower in preeclampsia patients compared to healthy pregnant women [18]. A TGF-β signaling molecule is associated with NO in the preeclampsia syndrome through the SMAD line [19],[20]. NO in normal pregnancy is produced by extravillous trophoblast, which has a vital function in remodeling the maternal spiral arteries to maintain a low placental resistance so that fetomaternal blood flow is well supplied [21],[22],[23]. In the condition of preeclampsia, there is a disturbance in the balance of angiogenic and antiangiogenic factors, thereby reducing the availability of TGF-β [3],[11]. Endothelial dysfunction in preeclampsia is caused by hypercholesterolemia and is characterized by increasing sFlt-1 and sEng level serum as antiangiogenic factors, and decreasing of TGF-B, VEGF, dan PlGF level serum as proangiogenic factors. Endothelial damage in preeclampsia can be repaired by administering statins which can make upregulation of endothelial NO synthase (eNOS), balance angiogenic factors, and finally improve endothelial health cells [12],[24],[25],[26].

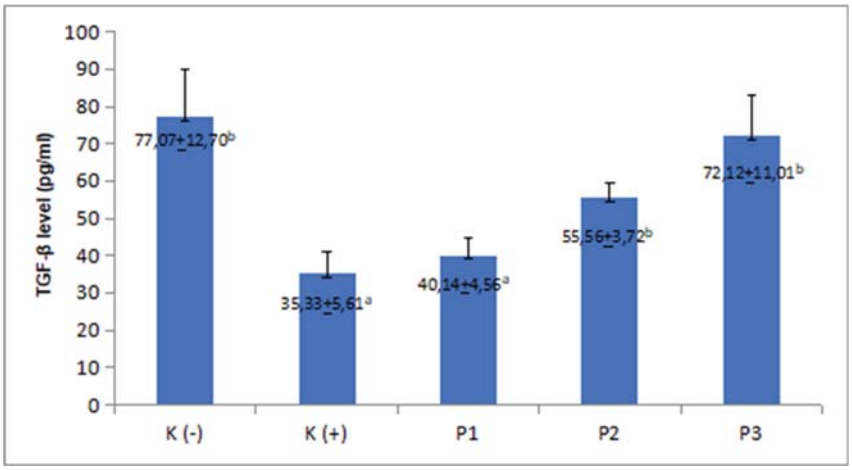

FIGURE 3. Effect of pravastatin on TGF-β serum levels in preeclampsia model rats. The one-way ANOVA test shows that there is a significant difference with p=0.003<α. LSD test result showed a significant difference between the two groups, which shows p =0.000. The statistical test shows that 8 mg/day is the optimum dose and a stronger positive correlation in increasing TGF-β levels in preeclampsia model Wistar rats (r=0.798; correlation is significant at the 0.01 level).

Pravastatin increases levels of TGF-β, and administration in each treatment group shows different abilities (Figure 3). The higher the dose is given results in higher the TGF-β level in the preeclampsia model of Wistar rats. The increase in TGF-β levels in the treatment group appeared to be followed by a decrease in the mean systolic BP and a decrease in urine protein [16],[27]. The endothelial layer of the glomerulus has a surface layer called the glycocalyx, which functions to prevent protein leakage. This layer also contains a lot of VEGF and TGF-β, which are produced by podocyte cells in the visceral layer. In preeclampsia, an increase in serum concentrations of sFlt-1 and sEng which causes a decrease in the concentration of VEGF, TGF-β causes an angiogenic imbalance so that endothelial cell health is disrupted and causes glomerular podocyte cell damage [1]. These findings are consistent with studies that showed the effects of pravastatin in the preeclamptic rat model in improving hypertension symptoms, urinary protein, and fetal condition [12],[13],[28],[29]. Pravastatin administered to a preeclampsia rat model with L-NAME show an increase in TGF-β, VEGF and PlGF followed by a decrease in sFlt -1 and sEng [11],[24],[28].

The positive correlation of the effect of pravastatin in increasing TGF-β in this study is shown by the higher the dose that results in a higher level of produced TGF-β. This study at a dose of 8 mg/day is the optimal dose that can increase TGF-β at the highest level. The administration of 2 mg/day shows a mean increase in TGF-β that is not significantly different in value than the positive control group. This condition indicates that the administration of pravastatin 2 mg/day can increase TGF-β levels but not so significantly that it requires higher doses to increase TGF-β levels. Pravastatin dose of 4 mg/day can increase TGF-β levels significantly, but a dose of 8 mg/day produces a closer mean value of negative control more than a dose of 4 mg/day. A dose of 8 mg/day in rats is equivalent to 40 mg/day in humans. This follows previous studies, which stated that a dose of 20-40 mg/day in humans can have an inhibitory effect on heart cholesterol by as much as 70-80%. Pravastatin can decrease cholesterol synthesis by inhibiting HMG-CoA reductase, thereby increasing binding to LDL receptors (LDLr) and decreasing plasma cholesterol [30]. Inhibition of HMG-CoA can decrease sFlt-1 and sEng. sFlt-1 is known to be an inhibitor of VEGF and PlGF, and sEng is also known to be an inhibitor of TGF-β in binding to their receptors to trigger endothelial dysfunction [3],[15], [25], [31]. Statins increase the expression of HMOX-1, an enzyme that has anti-inflammatory and antioxidant properties. Statin stimulation stimulates HMOX-1 to inhibit sFlt-1 and sEng, thereby reducing endothelial dysfunction by decreasing sFlt-1 and sEng. The regulation of sFlt-1 by statins appears mediated by inhibition of HMG-CoA reductase. Statins, through this mechanism, can reverse the endothelial dysfunction and angiogenic imbalance that underlie the pathophysiology of preeclampsia [11],[12]. Based on the above description, the investigators assume that the slightly increased mean TGF-β levels at 2 mg/day are due to L-NAME injection since the 13th day, which can inhibit the increase of TGf-β earlier so that when the TGF-β which should appear on the 13.5th day of pregnancy and the highest on the 19th day cannot reach normal TGF-β levels so that a larger dose of pravastatin is needed. It is proven that TGF-β levels increase at a dose of 4 mg/day and 8 mg/day, followed by a decrease in blood pressure and urine protein, but the optimum dose that can be optimum is 8 mg/day.

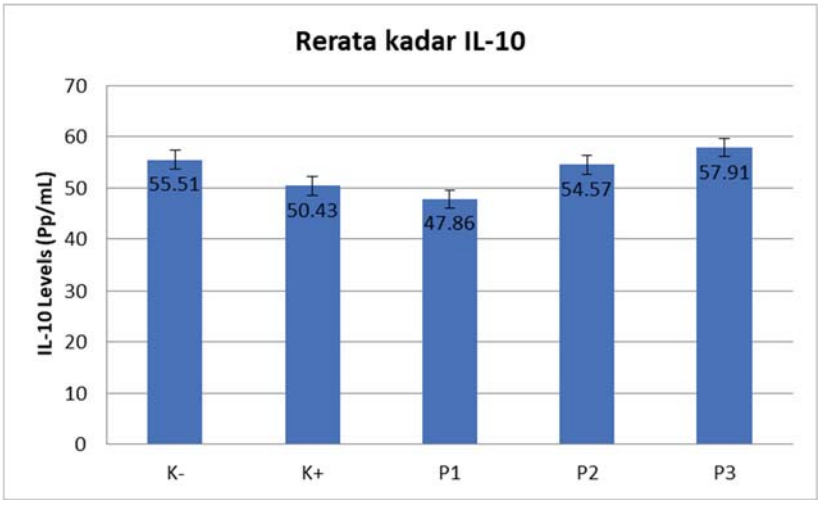

FIGURE 4. Effect of pravastatin on IL-10 serum levels in preeclampsia model rats. The one-way ANOVA test shows a significant difference with p=0.001<α. LSD test result shows that there is a significant difference between the two groups, which showed p=0.000. The statistical test shows that 8 mg/day is the optimum dose and a stronger positive correlation in increasing IL-10 levels in preeclampsia model Wistar rats (r=0.882; correlation is significant at the 0.01 level).

The levels of IL-10 in the negative control group are higher than the positive control injected L-NAME (Figure 4). It can be interpreted that the level of IL-10 in preeclampsia rats is lower than in the group of normal pregnant rats. The results of this study are in line with research that shows a decrease of IL-10 levels in preeclamptic pregnant women compared to normal pregnancy [7],[32]. IL-10 secretion plays a role in maintaining a normal pregnancy process [33]. IL-10 is an anti-inflammatory cytokine secreted by trophoblasts and T lymphocytes as an immunosuppressant in maintaining a normal pregnancy. The role of IL-10 during pregnancy is to suppress maternal immunity to allow acceptance of fetal allograft properties. IL-10 can increase the differentiation of Treg cells. IL-10 can downregulate proinflammatory cytokines such as interferon-γ (IFN-γ), IL-2 and TNF-α [34]. IL-10 is a cytokine that plays a role in the pathophysiology of preeclampsia [9],[35].

The doses of pravastatin that are considered successful in increasing IL-10 levels are 8 mg/day. The dose of pravastatin 8 mg/day is considered to increase IL-10 levels to reach Il-10 levels in normal pregnant rats. In general, it can be said that pravastatin can increase IL-10 levels in preeclampsia pregnant rats in this study. The results of this study are in line with Girardi, which reveals that pravastatin corrects the imbalance in Th1/Th2 cytokine response observed in preeclampsia [13]. D.D. Smith and M.M. Constantine also identify that statin can decrease inflammation [12]. It is characterized by a decrease of Th1 pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1, IL-2) and an increase of Th2 anti-inflammatory cytokines (IL-10, IL-4). IFN-γ plays an important role in the immune response by stimulating immune cells to express primary histocompatibility complex class II (MHC-II) proteins, which in turn activate T lymphocytes, so that Th2 anti-inflammatory cytokine can be increased, including IL-10. Pravastatin can increase IL-10 levels [13]. The increase in IL-10 causes a decrease in ET-1 and IFN-γ levels, aortic relaxation response, and a decrease in urinary protein in preeclampsia model mice [7],[8],[9],[36].

The administration of pravastatin dose 8 mg/day in experimental animals, which is equivalent to 40 mg/day of pravastatin in humans, successfully reduced systolic blood pressure, proteinuria, and decreased inflammation. This is reinforced by the correlation test results, which concluded that the higher the dose of pravastatin, the higher the level of IL-10 in the preeclampsia model rats in this study. Pravastatin has pleiotropic effects, including proangiogenic effects and anti-inflammatory effects [11],[12]. The increase of TGF-β levels and IL-10 levels can restore normal endothelial function.

A limitation of our study is that we did not observe the effects of pravastatin on a group of normal pregnant rats. We also did not give L-NAME at the beginning of implantation. We did not terminate at the time of early and mid-gestation. We could not distinguish the effect of pravastatin on early and late-onset as we knew in previous studies that early-onset and late-onset features would give a different picture in the pathophysiology of preeclampsia.

CONCLUSION

Pravastatin can increase TGF-β and IL-10 levels significantly in preeclampsia rat models.

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