Proceeding of The International Conference of Inovation, Science, Technology, Education, Children, and Health

E-ISSN: 2776-9062

Research Article

The Effect of Mulberry Leaves (Morus alba L) on Blood Pressure and Proteinuria in Rattus Norvegicus Wistar Strain Pre-Eclampsia Model

Tut Rayani Aksohini Wijayanti [1]*, Reny Retnaningsih [2], Rani Safitri [3]

[1,2,3] Bachelor of Midwifery Program, Faculty of Health Sciences, Institut Sains dan Teknologi Kesehatan RS dr. Soepraoen, Malang, Indonesia; Email: tutrayani@itsk-soepraoen.ac.id

* Corresponding Author : Tut Rayani Aksohini Wijayanti

Abstract: Preeclampsia is a leading cause of maternal morbidity and mortality globally, including in Indonesia. It is characterized by hypertension and proteinuria, which appear after 20 weeks of pregnancy. The pathophysiology of preeclampsia is closely linked to oxidative stress, which is caused by abnormal placentation. One promising alternative treatment for managing preeclampsia is the use of natural ingredients with antioxidant properties, such as mulberry leaves (Morus alba). These leaves contain flavonoids, such as rutin and quercetin, which are known to have antioxidant effects. This study aims to examine the effects of mulberry leaf extract on blood pressure and proteinuria levels in male Wistar rats, using a preeclampsia model. The study employed a true experimental post-test only control group design. A total of 25 pregnant rats were randomly divided into five groups: a negative control group, a positive control group (which was induced with suramin to model preeclampsia), and three treatment groups receiving mulberry leaf extract at doses of 12.5, 25, and 50 mg/kgBW. Blood pressure and proteinuria levels were measured before and after 6 days of treatment. The results indicated that mulberry leaf extract significantly reduced both systolic and diastolic blood pressure and markedly lowered proteinuria levels. A significant relationship was observed between blood pressure and proteinuria ($r = 0.528$; $p = 0.008$), suggesting that the reduction in blood pressure was associated with a decrease in proteinuria. These findings suggest that mulberry leaf extract may be a promising natural complementary therapy for alleviating symptoms of preeclampsia, offering an alternative treatment approach to help manage this serious pregnancy complication. Further studies are needed to explore its potential in clinical applications.

Keywords: Mulberry leaves; Preeclampsia; Proteinuria

1. Introduction

Preeclampsia is one of the leading causes of maternal morbidity and mortality worldwide. This condition is characterized by hypertension and proteinuria (excessive protein excretion in the urine) that develops after 20 weeks of gestation (Kathleen et al., 2012). According to Cuningham (2001), preeclampsia causes 50,000–70,000 maternal deaths each year, and is estimated to affect 5–8% of all pregnancies (Saftlas et al., 1990). In the United States, almost 18% of maternal deaths are related to preeclampsia (ACOG, 2002).

In Indonesia, this problem is also very serious. UNICEF data (2012) shows that Indonesia still ranks 3rd highest in maternal mortality (MMR) in the ASEAN region after Cambodia and Laos. Based on the report of the Ministry of Health (2014), around 27.1% of maternal deaths in Indonesia are caused by hypertension in pregnancy, including preeclampsia.

Physiologically, in normal pregnancy, mild oxidative stress occurs which can be controlled by the body's antioxidant system so that it does not develop into complications.

Received: 17, May 2025
Revised: 31, May 2025
Accepted: 16, June 2025
Published: 30, June 2025
Curr. Ver.: 30, June 2025

However, in preeclampsia, there is imperfect placentation, especially in the process of spiral artery remodeling and suboptimal trophoblast invasion. This condition causes decreased blood perfusion to the placenta, which leads to hypoxia/ischemia and increased oxidative stress (Hansson et al., 2015). This oxidative stress can occur both in the maternal circulation and in the placental tissue (Sheikhi et al., 2015). As a result, there is a decrease in relaxation factors and an increase in vasoconstriction factors, which causes placental ischemia, hypoxia, and the production of large amounts of free radicals (Sowinski, 2000; Goligorsky & Gross, 2001; Lidapraja, 2013).

In treatment efforts, the use of natural ingredients as complementary therapy is considered to have great potential because of its relatively low side effects compared to synthetic drugs. One of the natural ingredients that has antihypertensive potential is mulberry leaves (Morus alba). Research by Aminah et al. (2014) showed that mulberry leaves contain rutin and quercetin, two flavonoid compounds that have antioxidant effects and are beneficial to the cardiovascular system.

Due to its abundant availability, low price, and strong bioactive compound content, it is necessary to conduct research on the effects of giving mulberry leaf extract on blood pressure and proteinuria in an animal model of preeclampsia, namely Rattus norvegicus strain Wistar. This research is expected to provide an alternative natural therapy to help overcome preeclampsia.

2. Research Methods

2.1 Research Design

This study was conducted with a true experimental design model, post test only control group type, where the model animals will be divided into several treatment groups. There are 5 treatments for model animals, namely group A (negative control), group B (positive control), group C (suramin + mulberry leaf extract 12.5 mg / kg BW), group D (suramin + mulberry leaf extract 25 mg / kg BW) and group E (suramin + mulberry leaf extract 50 mg / kg BW). This study aims to determine the effect of giving mulberry leaves (morus alba l) on blood pressure and proteinuria in rattus norvegicus strain wistar preeclampsia model.

2.2 Sample

Samples were taken randomly from an affordable population, namely 10-week-old pregnant rattus norvegicus strain Wistar (according to the experimental age) located in the Experimental Animal Development Unit, Brawijaya University Malang, with the conditions in accordance with the inclusion and exclusion criteria.

2.2.1 Inclusion criteria

a. Body weight: 180-220 grams, healthy.
b. Physically healthy as indicated by active movement, clear eyes, shiny white fur that does not fall out, and soft feces.

c. Never been pregnant or experienced chemical treatment and intake

2.2.2 Exclusion criteria

a. Mice died due to treatment.

b. Behavior changes (does not want to eat, weak).

c. Mice give birth before termination time

2.3 Sample Calculation

The calculation of the minimum sample size uses the experimental sample size formula from freeder, where $(t-1)(r-1) \geq 15$, t: is the number of treatments and r: is the number of experimental animals in each treatment group. This study used 3 treatment groups and 1 control group, so $t = 5$, $(5-1)(r-1) \geq 15$ so that $r \geq 4.75$. The number of mice used was 5 for each group (3 treatment groups and 2 control groups) so that the total number of samples used in this study was 25 mice.

2.4 Treatment Group

In this study, the treatment group was divided into 5 groups randomly (K-, K+, P1, P2, and P3). Ten preeclamptic rats were used as controls and 15 rats were used as treatment groups with mulberry leaf extract administration at dose 1 (12.5 mg/kgBW) for group P1, dose 2 (25 mg/kgBW) for P2 and dose 3 (50 mg/kgBW) for group P3.

2.5 Research Variables

a. Independent variable: administration of mulberry leaf extract to the treatment group.

b. Dependent variable: systolic blood pressure, diastolic blood pressure, proteinuria levels

2.6 Research Procedure

a. Rats were weighed according to the inclusion criteria to obtain the desired subjects.

b. After sample selection, the model animals will be given an adaptation period of 1 week (7 days) with a standard diet to control the condition of the rats.

c. Randomization of model animals. Randomization was carried out to determine the control group and treatment group.

d. Model rats were injected with suramin to produce preeclampsia rats on days 9, 10, and 11 of pregnancy.

e. Treatment division.

f. Placement of research subjects in separate cages, daily feed consumption was measured.

g. On the 12th day, blood pressure and proteinuria were measured.

h. On the 12th day, rats were given treatment according to their treatment group (K-, K+ and P1, P2, P3) for 6 days.

i. On the 17th day of treatment, the model animals will have their blood pressure and proteinuria levels measured for research purposes.

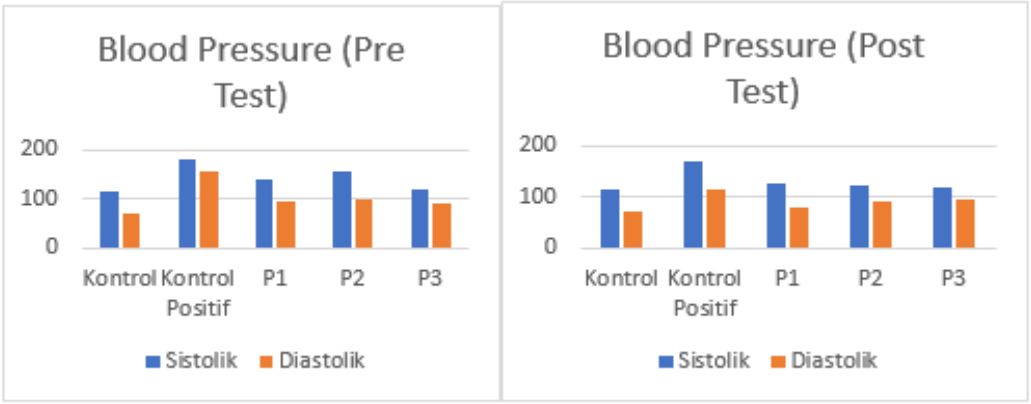
2.7 Data Collection

a. Calculation of daily feed consumption is done by weighing the initial weight and final weight of the feed. The results of the calculation will then be converted into nutritional value.
b. Measurement of rat body weight by weighing using a digital scale (electronic balance) with an accuracy of 0.1 g. Weighing is done at the beginning of maintenance in the laboratory until the last day of treatment.
c. Blood pressure using CODA® tail-cuff blood pressure system on days 12 and 17 d. Urine examination using reagent strips on urine samples on days 12 and 17.

3. Results and Discussion

3.1. Mulberry leaf extract therapy (Morus alba L) reduces systolic and diastolic blood pressure in rattus norvegicus strain wistar preeclampsia model.

Table 1. Pre-Test and Post-Test Blood Pressure Graph in Rattus Norvegicus Wistar Strain Preeclampsia Model



From the data in Table 1, it can be seen that there was a decrease in systolic and diastolic blood pressure in rats that had been injected with mulberry extract. This high blood pressure is a clinical symptom of preeclampsia. Blood pressure in the control group was 113.6/71.6 mmHg. In the positive control group (P0), the average systolic/diastolic pressure before being given mulberry leaf extract was 180.6/155.8 mmHg, decreasing to 170/115.4 mmHg. In group P1 with the administration of mulberry extract at a dose of 12.5 mg/kgBW, the average systolic/diastolic pressure also decreased from 140.2/95.4 mmHg to 125.6/80 mmHg. In group P2 with the administration of 25 mg/kgBW mulberry leaf extract, there was a decrease from 155.6/100.4 mmHg to 121.2/90.6 mmHg. In group P3, namely the group with a dose of 50 mg/kgBW mulberry extract, there was no significant decrease in the average systolic/diastolic pressure from 120.4/90.4 mmHg to 117.2/95.6 mmHg. The effect of suramin induction on trophoblasts of pregnant white rats can be seen through an increase in systolic and diastolic blood pressure. This inflammation can also be seen from the occurrence

of apoptosis (Gondo, 2016). The administration of suramin injection can actually cause inflammation in the trophoblast. The administration of suramin can actually cause preeclampsia. Preeclampsia that occurs will also be followed by clinical signs such as increased blood pressure (Table 4.1). VEGF levels also increase due to hypoxia. Hypoxia in pregnancy can occur due to failure of spiral artery remodeling and suboptimal trophoblast invasion due to symptoms caused by preeclampsia (Raghupathy, 2013).

Mulberry leaf extract can lower hypertension blood pressure because mulberry plants, especially the leaves, are diuretic (sweat-relieving). contains Mulberry phytosterogen which is also a flavonoid (Sutaryo, 2011). Flavonoids work as Angiotensin Converting Enzyme/ ACE inhibitors which inhibit the conversion of Angiotensin I to Angiotensin II so that peripheral vasodilation occurs, total resistance decreases and aldosterone secretion decreases which causes sodium and water excretion, and potassium retention, resulting in decreased Utilization of mulberry leaves blood pressure. in lowering blood pressure in hypertensive patients, namely by removing body fluids (through urine). Regular use of mulberry leaf decoction for 6 days can lower blood pressure, because mulberry leaves contain minerals, namely potassium, magnesium, and phosphorus. In addition, mulberry leaves are also diuretic because they contain a lot of water so they help lower blood pressure.

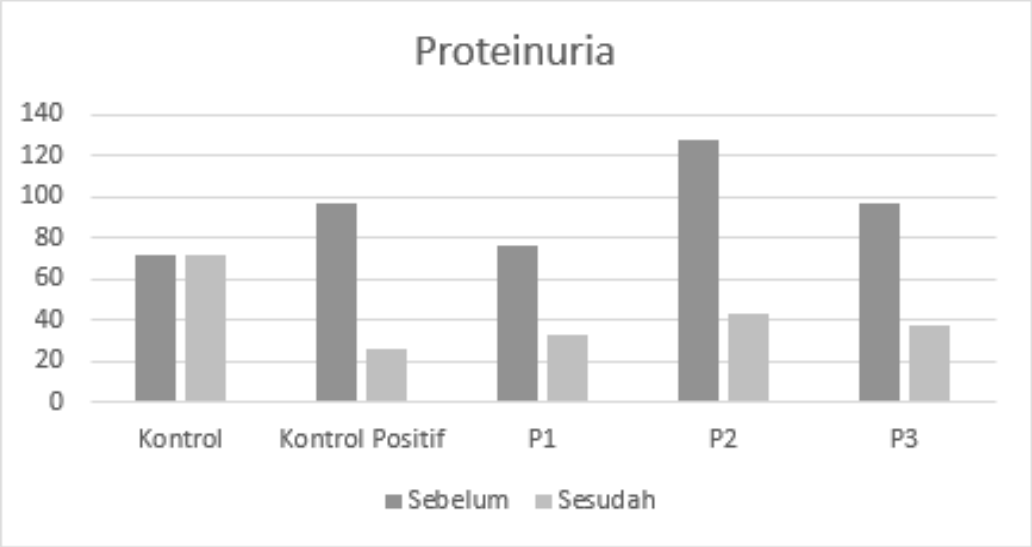
Mulberry leaf extract can lower blood pressure due to the healing power of mulberry leaves which are diuretics, one of the functions of which is to remove a number of fluids and electrolytes as well as toxic substances. By reducing the amount of water and salt in the body, the blood vessels will loosen so that blood pressure will slowly decrease.

The results of this study are in accordance with the theory written by Hidayat (2015) in the book Kitab Tumbuhan Obat & Khasiatnya that mulberry leaves contain phytosterogens that work as flavonoids and also contain diuretics that work by removing a number of fluids and electrolytes as well as toxic substances. By reducing the amount of water and salt in the body, the blood vessels will loosen so that blood pressure will slowly decrease.

The results of this study indicate that mulberry leaf extract is an alternative to lowering blood pressure because of the effects of flavonoids and is diuretic so that it can lower blood pressure. Flavonoids work as an Angiotensin Converting Enzyme/ ACE inhibitor which inhibits the conversion of Angiotensin I to Angiotensin II so that vasodilation occurs, total peripheral resistance decreases and aldosterone secretion decreases which causes sodium and water excretion, as well as potassium retention, resulting in a decrease in blood pressure.

3.2. Mulberry leaf extract therapy (Morus alba L) reduces proteinuria levels in rattus norvegicus strain wistar preeclampsia model.

Table 2. Pre-Test and Post-Test Graph of Proteinuria in Rattus Norvegicus Wistar Strain Preeclampsia Model



From the data in Table 2, it can be seen that there was a decrease in proteinuria in rats that had been injected with mulberry extract. This high proteinuria is a clinical symptom of preeclampsia.

Proteinuria in the control group was 71.6. In the positive control group (P0), the average proteinuria before being given mulberry leaf extract was 97.2, decreasing to 25.7. In group P1 with a dose of 12.5 mg/kgBW mulberry extract, the average proteinuria also decreased from 77.04 to 32.8. In group P2 with a dose of 25 mg/kgBW mulberry leaf extract, it decreased from 127.92 to 43.68. In group P3, namely the group with a dose of 50 mg/kgBW mulberry extract, there was a decrease in the average proteinuria from 97.02 to 38.02.

The urine sample used for this examination was morning urine. Urine pH examination was carried out using a universal pH indicator, based on the results of pH observations showed that the normal group and the test group had the same urine pH, which was 5. Normal urine has an acidic pH, which is in the range of 5.5-8 (DN. Baron, 1990). The relationship between pH and kidney function is because the kidneys are important organs in maintaining body homeostasis (Tjay, 2007), one of which is maintaining pH balance (Corwin, 2009). Therefore, the urine pH of mice in the normal group, dose group 1 and dose group 2 showed normal urine pH. Urine protein examination was carried out using the dipstick method or dipstick using a stick dipped into the urine and the reading was done by comparing the color results of the stick on the standard dipstick table. The proteinuria test is a preliminary examination to determine early symptoms of kidney damage (Stein, 1998). A positive result of one (+) indicates the presence of protein that exceeds the normal limit in the urine and traces are considered normal (Schwartz, 2004). One of the most common causes of

proteinuria is functional causes, which can occur in normal kidney conditions but the increase in proteinuria is influenced by a temporary increase in protein excretion due to certain conditions. The factor that most influences this condition is protein intake in mouse feed, where the protein content of the feed is 17.5-19.5% per kilogram. Under normal conditions, the glomerular membrane only allows low-molecular proteins to be filtered and reabsorbed or catabolized by cells in the proximal tubule and holds back macromolecular filtration such as albumin protein and then excretes a small amount of unfiltered protein (Sylvia, 2005). So that a fairly high protein content in the feed will increase the excretion of albumin protein in mouse urine which can be detected in a dipstick test, this is a functional cause of proteinuria.

3.3. Effect of giving mulberry leaves (Morus alba L) on blood pressure and proteinuria in rattus norvegicus strain wistar preeclampsia model

Table 3. The effect of giving mulberry leaves (Morus alba L) on blood pressure and proteinuria in Rattus Norvegicus Wistar strain preeclampsia model

Variable Relationship	Correlation Coefficient	p-value	Information
Blood pressure with Proteinuria	**0,528**	**0,008**	**Signifikan**

The results of the analysis in Table 3 show a significant relationship between blood pressure and proteinuria in preeclampsia model rats after administration of mulberry leaf extract (Morus alba), with a correlation coefficient of 0.528 and a p value = 0.008. A p value smaller than 0.05 indicates that the relationship between the two variables is statistically significant.

A positive correlation coefficient indicates that the higher the blood pressure, the higher the level of proteinuria, and vice versa. This is in accordance with the pathophysiological mechanism of preeclampsia, where impaired placental perfusion due to vasoconstriction and oxidative stress causes increased blood pressure and glomerular endothelial damage, leading to protein leakage in the urine (Hansson et al., 2015; Sheikhi et al., 2015).

Administration of mulberry leaf extract can provide positive effects because it contains flavonoids, especially rutin and quercetin, which have been shown to have antioxidant and antihypertensive activities. Flavonoids work by neutralizing free radicals, increasing vasodilation through nitric oxide (NO) activation, and reducing oxidative stress which is one of the main causes of endothelial damage in preeclampsia (Aminah et al., 2014; Peluso, 2006).

By decreasing oxidative stress and improving endothelial function, there is a decrease in blood pressure and improvement in glomerular membrane integrity, which ultimately reduces protein excretion in the urine. This explains the significant correlation between decreased blood pressure and decreased proteinuria after administration of mulberry leaf extract.

These findings support the results of previous research by Lee et al. (2010) which reported that mulberry leaf extract is able to lower systolic blood pressure and improve endothelial function through strong antioxidant activity.

4. Conclusions

The administration of mulberry leaf extract (Morus alba L) to Rattus norvegicus strain Wistar rats with preeclampsia model showed a significant relationship between decreased blood pressure and decreased proteinuria levels, with a correlation coefficient of 0.528 and a p value = 0.008. This indicates that mulberry leaf extract has the potential as a natural antihypertensive and antiproteinuria agent through an antioxidant mechanism that can reduce oxidative stress, increase vasodilation, and improve endothelial function.

These results support the use of mulberry leaves as a complementary therapy in treating preeclampsia, because it is easily obtained, affordable, and has minimal side effects.

References

Ahmed, A., Ahmad, S., Hewett, P. W., & Al-Ani, B. (2011). Autocrine activity of soluble Flt-1 controls endothelial cell function and angiogenesis. *Vascular Cell, 3*, 15. https://doi.org/10.1186/2045-824X-3-15

Cindrova-Davies, T., Sanders, D. A., & Burton, G. J. (2011). Soluble FLT1 sensitizes endothelial cells to inflammatory cytokines by antagonizing VEGF receptor-mediated signalling. *Cardiovascular Research, 89*(3), 671-679. https://doi.org/10.1093/cvr/cvq346

Davis, E. F., Newton, L., Lewandowski, A. J., & Lazdam. (2012). Pre-eclampsia and offspring cardiovascular health: Mechanistic insights from experimental studies. *Clinical Science, 123*(2), 53-72. https://doi.org/10.1042/CS20110627

Giachini, F. R., Galaviz-Hernandez, C., & Damiano, A. E. (2017). Vascular dysfunction in mother and offspring during preeclampsia: Contributions from Latin-American countries. *Current Hypertension Report, 19*(10). https://doi.org/10.1007/s11906-017-0781-7

Gondo, H. K. (2016). Efek protektif phycocyanin terhadap preeklampsia melalui hambatan jalur inflamasi, stres oksidatif dan apoptosis sel trofoblas. *Universitas Brawijaya: Malang.*

Hidayat, S., & Rodame, M. N. (2015). *Kitab Tumbuhan Obat*. Agriflo.

Indrawan, I. (2017). Peranan ekstrak etanol biji jinten hitam menurunkan kadar IL-1b, IFNg, endothelin-1 mencit model preeklamsia melalui penghambatan aktivasi p65. *Program Studi Doktor Ilmu Kedokteran Fakultas Kedokteran Universitas Brawijaya*.

Kvehaugen, A. S., Dechebd, R., Ramstad, H. B., & Troisi, R. (2011). Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. *Hypertension, 58*(1), 63-69. https://doi.org/10.1161/HYPERTENSIONAHA.111.172387

Matsubara, K. (2017). Hypoxia in the pathogenesis of preeclampsia. *Hypertension Research in Pregnancy, 5*(2), 46-51. https://doi.org/10.14390/jsshp.HRP2017-014

Myrank. (2009). *Bebas hipertensi tanpa obat*. Agromedia.

Nugraheny, D., Permatasari, N., & Saifur Rohman, M. (2017). Physalis minima leaves extract induces re-endothelialization in deoxycorticosterone acetate-salt-induced endothelial dysfunction in rats. *Research Journal of Life Science, 4*(3), 199-208. https://doi.org/10.21776/ub.rjls.2017.004.03.6

Ornaghi, S., & Paidas, M. J. (2014). Upcoming drugs for the treatment of preeclampsia in pregnant women. *Expert Review of Clinical Pharmacology, 7*(5), 599-603. https://doi.org/10.1586/17512433.2014.944501

Parsanezhad, M. E., Attar, A., Namavar-Jahromi, B., & Khoshkhou, S. (2015). Changes in endothelial progenitor cell subsets in normal pregnancy compared with preeclampsia. *Journal of the Chinese Medical Association, 78*(6), 345-352. https://doi.org/10.1016/j.jcma.2015.03.013

Raghupathy, R. (2013). Cytokines as key players in the pathophysiology of preeclampsia. *Medical Principles and Practice, 22*(suppl 1), 8-19. https://doi.org/10.1159/000354200

Robinson, M. B., & Sasodo, J. (2005). Pekan ulat sutra morus. *[Online]. Available: http://lpi.oregonstate.edu/infocenter/phytochemicals/garlic/#table1*

Salman, S., Kumbasar, S., & Yilmaz, M. (2011). Investigation of the effects of the chronic administration of some antihypertensive drugs on enzymatic and non-enzymatic oxidant/antioxidant parameters in rat ovarian tissue. *Gynecological Endocrinology, 27*(11), 895-899. https://doi.org/10.3109/09513590.2010.551564

Sipos, P. I., Crocker, I. P., & Hubel, C. A. (2010). Endothelial progenitor cells: Their potential in the placental vasculature and related complications. *Placenta, 31*(1), 1-10. https://doi.org/10.1016/j.placenta.2009.10.006