Effect of Mahkota Dewa Ethanolic Extract (*Phaleria macrocarpa*) to Kidney Histology of Preeclampsia Rats

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Abstract. Hypertension is an increase in systolic and diastolic pressure, the height of which depends on the age of the individual affected. Hypertension can also cause terminal kidney failure (GGT). One of the herbs that has the effect of reducing blood pressure is the mahkota dewa. This study aims to determine the effect of mahkota dewa extract on histological and morphological features of the kidneys in preeclampsia rat. In this study using a type of analytic research with quasi-experimental design in pregnant rat (*Rattus novergicus*) as many as 24 rats and consisted of 4 treatments (each treatment was divided into 6 rats). control group, P1 (pregnant rats injected by LPS) P2 (pregnant rats injected with LPS given 100 mg / BW of mahkota dewa extract once a day) and P3 (pregnant rats injected with LPS and given 200 mg/BW of deity extract twice a day). On the 17th day the pregnancies of the rat were dissected and taken by kidney organs and then made a paraffin method preparation using Hematoxylin-Eosin (HE) staining. Morphological observations showed a change in color in group K (control) = blackish red, at P1 = pale red, P2 = brownish red and P2 = thick red While the results of kidney histology on tubular narrowing and necrosis obtained significant results (P <0,05) between P1 and P3. This shows that the mahkota dewa is very influential on kidney damage in preeclampsia models.

1. Introduction

Preeclampsia and hypertension is one of the causes of maternal and infant mortality, with a fairly high incidence in Indonesia. Preeclampsia often occurs in the first pregnancy and occurs in women who previously suffered from high blood pressure. Preeclampsia is defined generally as hypertension and proteinuria that occur after 20 weeks of pregnancy [1].

Hypertension is an increase in systolic and diastolic pressure, the height of which depends on the age of the individual affected. Hypertension can also cause terminal renal failure (GGT) through a process that can result in the loss of a large number of progressive functional nephrons. The severity of the influence of hypertension on the kidneys is very dependent on the high blood pressure and the length of a person suffering from hypertension [27]. The higher the blood pressure for a long time, the more severe complications that can be caused [2,18-20].

Treatment of high blood pressure can be done by pharmacological and nonpharmacological methods. Nonpharmacological treatment is carried out by controlling hypertension, such as managing a healthy diet and lifestyle. While pharmacological treatment can be done by administering diuretic or...
vasodilator drugs. The use of herbal plants has proven healthy and does not cause side effects [15, 17, 25, 28]. One of the herbs that has the effect of reducing blood pressure is the mahkota dewa. Giving mahkota dewa fruit once a day for 7 days can reduce blood pressure in patients with hypertension [3].

The development and utilization of mahkota dewa plants for healing various diseases such as kidney, hypertension, liver and heart, the greater the potential in the community. This is due to the safety and efficacy that has been proven empirically. However, the research on the mahkota dewa is scientifically not yet proven its safety. Therefore, researchers are interested in conducting research on mahkota dewa plants which are thought to affect the kidneys of someone suffering from hypertension [4].

2. Materials and Methods

2.1. Research Design
This study used a type of analytical study with an experimental quasi design in pregnant female rats (Rattus norvegicus). The technique or method of grouping is by editing 4 groups of rats divided into 4 treatments, namely: group 1 ie (normal) control in the form of rat that are pregnant not given any treatment, only given excessive feed and drink (ad libitum). Group 2, namely P1 in the form of pregnant rat injected with LPS (lipopolysaccharide) to become a model mouse for preeclampsia.

Group 3 is P2 pregnant rat injected with LPS (lipopolysaccharide) and given mahkota dewa extract (Phaleria macrocarpa) at a dose of 100 mg / kgBW once a day. Group 4 is P3 in the form of pregnant rat injected with LPS (lipopolysaccharide) and given mahkota dewa extract (Phaleria macrocarpa at a dose of 200 mg / kgBW twice a day with 6 replications according to the Fereeder formula, namely:

$$\text{Information :}$$

$$t = \text{Number of treatment groups}$$

$$n = \text{Number of replications}$$

| Treatment | Control | LPS Injection | Mahkota Dewa Ethanolic Extract (Phaleria macrocarpa) |
|-----------|---------|---------------|-----------------------------------------------------|
| K         |         |               | 100 mg/Kg BW                                        |
| P1        | √       |               | 200 mg/Kg BW                                        |
| P2        | √       | √             |                                                     |
| P3        | √       | √             |                                                     |

2.2. Sample Preparation
The extraction method used was maceration with ethanol solvents [10]. Making extracts is done by soaking the simplicia for 48 hours in 50% ethanol and shaken regularly. Then filtered using Whatmann paper and carried out evaporation to separate the solvent with extraction results at a temperature of 18-32°C

2.3. Acclimatization of Experimental Animals
Female rats (Rattus norvegicus), healthy, and 10 weeks of age with a weight of 150-250 grams as many as 24 birds. Rats were divided into the control and treatment groups. rat are given excessive food and drink (ad libitum), and are kept in a clean cage with sufficient moisture and light. Handling rat according to the experimental animal code of ethics (Ethical clearance from the University of Sumatera Utara Faculty of Mathematics and Natural Sciences Animal Research Ethics Commission).
2.4. Injecting LPS and Giving Extract
Rat were given pellet feed and drank excessively (ad libitum), then rat were injected with LPS through intervena on the tail to become a model mouse for preeclampsia. After that, proteurinaria is checked daily and systolic blood pressure monitoring is carried out once every three days in rat injected with LPS then given the mahkota dewa extract (*Phaleria macrocarpa*) orally at a dose of 100 mg / kgBW and 200 mg / kgBW. Giving extracts was carried out orally using gavage needles and 1 ml syringe.

2.5. Organ Harvesting and Preparation of Preparations
Anesthetized rat using chloroform, then dislocated neck after anesthetic conditions were surgically performed on the abdomen. After the abdominal cavity is opened, the kidneys are taken and inserted into the sample bottle which contains 10% Formalin Neutral Buffer. The making of histological preparations is carried out using a 10% Formalin Neutral Buffer solution and then cut and inserted into a cassette or specimen location. Then the dehydration process is carried out on alcohols with multilevel concentrations, 70% alcohol, 96% and absolute alcohol. Then it is done by xylol purification and then printed using paraffin so that the preparation is printed in paraffin blocks and stored in the refrigerator. The paraffin block is then thinly cut into 5-6 thickness using a microtome. The results of the pieces are floated in warm water at 60°C to stretch so the tissue does not multiply. The preparation is then lifted and placed on a glass object to do Hematoxylin and Eosin (HE) staining. Furthermore, it was observed under a microscope with 400x magnification [24].

2.6. Test Parameter Analysis

2.6.1. Visual Kidney Morphology Analysis
Rats were surgically removed in each treatment and the kidneys were taken and then weighed by each organ using a balance sheet and recorded. Observation of kidney color and compared to each treatment were carried out. The normal assessment of kidney color is a brownish-red surface, while abnormal spots and spots indicate color changes.

2.6.2. Kidney Histological Analysis
The kidneys are taken to make histopathological preparations using the paraffin method. Histopathological studies were carried out under a microscope with a magnification of 40 x10, Renal histological preparations were observed under a light microscope in 5 different visual fields, with a magnification of 40x10 times. Each field counted 20 cells randomly so that in one preparation 100 kidney cells were found. Then the weighted scores of renal histopathological changes in five fields of each rat with the Manja Roeni Histopathology Scoring model were calculated. The kidney structure observed was necrosis and narrowing of the kidney tubules.

2.6.3. Data Analysis
Data will be presented in the form of a mean standard deviation if the distribution is normal. If the distribution is not normal, the data will be presented in quartile form. Data was processed and analyzed using SPSS with a significance limit of p<0.05. to assess whether the sample was normally distributed or not conducted the Shapiro-Wik test because of the sample 50. To assess the intergroup parameters, the ANOVA test was used to collect normally distributed data and the Kruskal Wallis test data was abnormally distributed.

3. Results and Discussion
The results of this study include observations morphologically seen from the color of the kidneys. While histological observations include tubular narrowing and necrosis/apoptosis (cell death)
3.1. Kidney Color
The color of female kidney (*Rattus norvegicus*) after the administration of ethanol extract of the fruit of the mahkota dewa (*Phaleria macrocarpa*) Model of preeclampsia can be seen as follows:

![Kidney Color Images](image.png)

Figure 1. Color of female rats (*Rattus norvegicus*) preeclampsia model after administration of mahkota dewa extract (*Phaleria macrocarpa*) K = Control (pregnant female without any treatment), P1 = Giving LPS in female rat, P2 = Extract of mahkota dewa (*Phaleria macrocarpa*) as much as 100 mg / kgBW, and P2 = Provision of mahkota dewa extract (*Phaleria macrocarpa*) as much as 200 mg / kgBW.

In the picture there are differences in kidney color, in group K (control) = blackish red kidney color (bright black), at P1 (LPS) = red kidney color is slightly pale, P2 (LPS + 100 mahkota dewa extract) = red kidney color is little chocolate and P2 (LPS + 200 mg mahkota dewa extract) = thick red. The mean renal size in red rat, but there were differences in kidney size seen in P2 group. Changes in kidney size increase with increasing doses of the mahkota dewa extract. This might happen because the chemicals contained in the mahkota dewa are excessive resulting in the kidney glomerulus working hard in filtering substances that enter the body. According to the study [9] the most common damage is damage that occurs in the urinary system. The kidneys are the main organ to get rid of metabolic waste products that are no longer needed by the body. Therefore, it is necessary to observe changes in morphological structure of rats in preeclampsia rats after administration of mahkota dewa extract.

3.2. Tubular Narrowing
Based on statistical data, there was a significant effect of crown dewa extract (*Phaleria macrocarpa*) between P1 and P3. This shows that the mahkota dewa is able to reduce the occurrence of narrowing of renal tubules (*Rattus norvegicus*) preeclampsia. In group P1 there was severe tubular narrowing damage compared to group P3. This is thought to be due to the chemicals contained in the extract of the mahkota dewa forming antibodies in response to the presence of antigens in the body that cause the formation of complex antigens which are involved in glomerular lumps or in a few cases this antigen builds up on the glomerular capillary walls causing inflammation and making the glomerulus cannot work properly so there is a narrowing of the tubules. According to the study [5] the kidney component has a very close relationship, the glomerulus and tubules form a kidney unit, changes in the glomerulus will result in changes in the kidney tubules and vice versa.
Figure 2. Narrowing of renal tubules (Rattus norvegicus) female models of preeclampsia after administration of mahkota dewa extract (*Phaleria macrocarpa*) K = Control (pregnant women without any treatment), P1 = Giving LPS in female rat, P2 = Extracting mahkota dewa extract (*Phaleria macrocarpa*) as much as 100 mg / kgBW, and P2 = 200 mg / kgBW extract of mahkota dewa (*Phaleria macrocarpa*)

Figure 3. Narrowing of renal tubules of female (*Rattus norvegicus*) models of preeclampsia (magnification 400x).

3.3. Necrosis

Based on the data above there are significant differences between P1 and P3 (p<0.05). This shows that there is an influence of mahkota dewa extract on renal cell death in preeclampsia rat. Necrosis is the death of cells and tissues in the living body. In necrosis changes appear markedly in the nucleus (cell nucleus). Cell death is characterized by shrinking the cell nucleus or cell nucleus inactivity [21-23, 29]. According to the study [4], necrosis begins with morphological changes in the cell's nucleus, namely pyknosis. The next stage is the broken core (karyorrhexis) and the core disappears. Nucleus morphology in necrosis consists of 3 patterns, namely:

a. Pyknosis, characterized by shrinking the cell nucleus and increasing basophils then the DNA condenses into a solid constricted mass.

b. Karyorrhexis, the fragment of the cell nucleus that is hypnotic, which in the next 1-2 days the nucleus in the dead cell completely disappears.

c. Karyolysis, characterized by the nucleus dying and disappearing caused by Deoxyribonuclease (DNase) activity.
Figure 4. Necrosis of female rats (Rattus norvegicus) preeclampsia model after administration mahkota dewa extract (Phaleria macrocarpa) K = Control (pregnant female without any treatment), P1 = Giving LPS in female rat, P2 = Extract of mahkota dewa (Phaleria macrocarpa) as much as 100 mg / kgBW, and P2 = Provision of mahkota dewa extract (Phaleria macrocarpa) as much as 200 mg / kgBW

4. Conclusion
Mahkota Dewa extract against blood pressure, and also kidney histology containing tubular narrowing and necrosis proved to have significant differences between control settings and P1. It is expected that from the color of the kidneys, color changes occur. The color in the control settings is red, in the P1 setting it is pale red, and the P2 is brownish red.

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