Effect of Mahkota Dewa Ethanolic Extracts (Phaleria macrocarpa Scheff.) to Hepar Histology of Female Rat (Rattus norvegicus) In Preeclampsia

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Abstract. Preeclampsia is a disease at 20 weeks gestation which is characterized by an abnormal increase in blood pressure (Hypertension) and Proteunaria. This study used a type of analytic study with an experimental quasi design in pregnant female rats (Rattus norvegicus) consisting of four treatments with six replications each. The study consisted of a control group (K) which was only fed ad libitum, Treatment 1 (P1) was injected with LPS intervena at a dose of 0.2 / kgBW, and the group was given extract of Mahkota Dewa Plant (Phaleria macrocarpa Scheff.) With each dose -one of 100 mg / kgBW (P2) and 200 mg / kgBW (P3). Rat liver organ (Rattus norvegicus) is taken after surgery. Liver organ is made into a preservative preparation with paraffin method and staining of Hematoxylin-Eosin (HE).

Morphological observations showed a marked decrease in liver weight (p <0.05) in P1 compared to Control and a change in liver color in P1 compared to controls. Normal hepatocyte histological observations and necrosis showed that there were significant differences (p<0.05) and hepatocyte parenchymatic degeneration and hydropic degeneration showed no significant differences (p>0.05).

1. Introduction
Preeclampsia is a disorder during pregnancy that occur after 20 weeks of gestation. Preeclampsia is generally characterized by an increase in blood pressure (hypertension) characterized by blood pressure above 140 mmHg systolic or 90 mmHg and proteunaria 0.3 gram / day. According to the World Health Organization (WHO), there are around 585,000 deaths among pregnant women, 58.1% of which are caused by preeclampsia and eclampsia [1-4]. Liver is the main organ as a defense against toxic. The liver is an organ that functions in the metabolism of excretion in the body [5]. Morphologically the liver is a simple organ but functionally so complex [6-7]. Efforts to prevent preeclampsia can be done by consuming vitamins C and B but so far these vitamins are considered to be less effective. Prevention of preeclampsia can also be done by taking antihypertensive drugs in the form of nifedipine, anti-seizures in the form of magnesium sulfate and methyldop. Utilization of these herbs based on practices that are inherited in descending ways [8-11]. One of the plants that is utilized is, among others, the god’s crown plant. The god’s crown plant is a plant that has active substances in the form of alkaloids, saponins and flavonoids. According to the study [12], the content contained in god’s crown plants has antioxidant effects and various properties [13-14], among others, can prevent...
hypertension. Based on the description above, this study was conducted to determine the effect of giving the god’s crown ethanol extract (Phaeria macrocarpa Scheff.) to the liver histological picture of preeclamptic female (Rattus norvegicus) rats.

2. Methods

2.1. Research Design
This study used the Complete Randomized Design Method which was divided into 4 treatments with 6 replications in accordance with the Federer formula:

\[(t-1)(n-1) \geq 15\]

Description:
\(t = \) treatment group
\(n = \) replications

The following treatment groupings can be seen in table 1.

| Treatment     | Control | LPS | Ethanolic extract of Phaleria macrocarpa |
|---------------|---------|-----|----------------------------------------|
|               |         |     | 100 mg/kg BW | 200 mg/kg BW |
| K             | √       |     |             |               |
| P1            |         | √   |             |               |
| P2            |         | √   |             | √             |
| P3            |         |     |             | √             |

2.2. Sample preparation and preparation of extracts
The extraction method used was maceration with ethanol solvents. Extracts is done by soaking the simplicia for 48 hours in 50% ethanol and shaken regularly then filtered using Whatmann paper and evaporation to separate the solvent with the extraction results at a temperature of 18-32°C [15-22].

2.3. Experimental and ethical issue of animal handling
Female rats were healthy, 10 weeks old with a weight of 150-200 grams as many as 24 head. Rats were divided into the control and treatment groups. Rats are given excessive food and drink (ad libitum), and are kept in a clean cage with sufficient moisture and light. Handling rats according to the experimental animal code of ethics (Ethical clearance from the Universitas Sumatera Utara, Faculty of Mathematics and Natural Sciences - Animal Research Ethics Commission).

2.4. Injecting LPS and extract administration
Rats were given pellet feed and drunk excessively (ad libitum), then rats were injected with LPS through intervenal on the tail to become a model rats for preeclampsia. After that, proteurinaria is checked every day (at 08-10) and monitoring systolic blood pressure every three days rats injected with LPS then given the god’s crown extract orally at a dose of 100 mg / kgBW and 200 mg / kgBW. Extract was given orally using gavage needles and 1 ml syringe [23-27].

2.5. Preparation of a Liver Surgery Preparation
Rats were anesthetized using chloroform, then dislocated the neck under anesthetic conditions were surgically performed on the abdomen. After the abdominal cavity is opened, the liver is taken and placed 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. 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 preparate is printed in paraffin blocks and stored in the refrigerator. Paraffin blocks are then thinly cut into 5-6 μm 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 [28-30].

2.6. Histopathological Analysis of the Liver

The liver was taken to make histopathological preparations using paraffin method. Histopathological studies were performed under a microscope with a magnification of 400x. Liver histological preparations were observed under a light microscope in 5 different fields, with a magnification of 40x10 x. Each field counted 20 cells randomly so that in one preparation 100 liver cells were found. Then the mean score of histopathology liver change score was calculated in five fields of each - each mouse with the Scoring Histopathology Manja Roenigk model. The liver structure observed was normal hepatocytes and hepatocytes which suffered damage both necrosis, parenchymous degeneration and hydropic degeneration. Then the number of percentages that occur [31] are counted and calculated. The Assessment of Hepatocyte Damage Assessment Criteria for Spoiled Roenigk who have been modified can be seen in Table 2.

Tabel 2. Assessment of Hepatocyte Damage Assessment Criteria for Spoiled Roenigk who have been modified

| Damage Rate          | Score |
|----------------------|-------|
| Normal               | 1     |
| Parenkimatosa Degeneration | 2     |
| Hydropic Degeneration | 3     |
| Necrosis             | 4     |

2.7 Data analysis

Data will be presented in the 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 is normally distributed or not, the Shapiro-Wilk test is done because the sample is ≤ 50. ANOVA test is used to evaluate the intergroup parameter comparison when the data is normally distributed and the Kruskal Wallis test if the distributed data is not normal.

3. Results and Discussions

3.1. Weight of Rat Liver

The results of the observation on the weight of the liver organs of the rats with the control treatment, LPS and injection of ethanol extract of god’s crown fruit the average heart weight in rats included K (9.8 g), P1 (8.0 g), P2 (8.7 g) and P3 (9.6 g) (Figure 1).
The results of statistical analysis show that there is a significant difference between control (K) and treatment (P1), which is a decrease in weight because of the LPS compound (lipopolysaccharide). According to the study [32], the use of toxic substances or chemicals will pose a risk for the body, one of which is liver, the liver is one organ that is susceptible to toxic or chemical substances. Toxic substances or chemicals that enter the liver will undergo metabolism which will reduce liver toxicity.

3.2. Description of Morphology of the Color and Surface of the Liver
The results of the observations on the morphology of the liver of rats that had been injected with LPS and given ethanol extract of god’s crown can be seen in Figure 2.
Control Treatment (Control without injected LPS) liver looks normal with brownish red color and non-grainy surface and liver P1 (LPS injection) looks abnormal with pale colors and freckles. Whereas in P2 and P3 (LPS + ethanol extract), the liver with brownish red and non-grainy surface was seen because the ethanol extract of the god’s crown plant was able to repair liver cells that had been injected by LPS. According to the study [32], biological natural materials that are able to prevent disease, one of which is the crown god plant. Empirically, the god crown plant contains flavanoid compounds which function for the treatment of liver cirrhosis and liver disease.

3.3. Histology of the liver

The results of observations made on the liver histology of pregnant rats treated with LPS and god’s crown ethanol extract can be seen in Figure 3.

Figure 3. Histological observations of female rats found cell changes. Notes; K = Control, P1 = LPS injection, P2 = LPS + ethanol extract (100 mg/kg BW), P3 = LPS + ethanol extract (200 mg/kg BW).

In Figure 3 shows the histological observations of female rats found cell changes. The percentage of normal cell hepatocytes in group K is more than P1. While the number of cells that experience swelling, hydroptic and necrosis is found in P1. This is probably due to the influence of the LPS compound (lipopolysaccharide). According to the study [33], liver damage is closely related to bleeding. Hepatocytes are polihedral cells that have a surface of six or more with clear cell membranes and a round core in the center. According to the study [31], parenchymal degeneration occurs due to oxidation failure which results in the transport of proteins that have been disturbed by ribosomes, resulting in accumulation of water in cells and causing cells to swell. According to the study [34], necrosis is death of the liver cells and is an acute damage. Some substances have been shown to cause liver necrosis. Necrosis is a dangerous toxic. Necrosis is the rupture of the plasma membrane [35-38].
4. Conclusions
The conclusions from this study are:

a. Giving LPS and ethanol extract of Mahkota Dewa plant can significantly affect the weight and surface of pregnant rat hearts (p<0.05).

b. The level of damage to the liver occurred in the LPS treatment and protection of hepatocyte damage occurred in the injection of ethanol extract of god’s crown (p<0.05).

c. Giving ethanol extract of god’s crown was able to repair hepatocytes damaged by preeclampsia of pregnant rats.

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