Histopatological Description Of Liver And Kidney Of Mice Exposed To The Ethanol Extract Of Syzygium Myrtifoliumwalp Leaves

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Abstract

Aimed to assess the toxic effects of ethanol extract of red shoot leaves to the histopathological picture of liver and kidneys of mice (Mus musculus). The treatment groups consisted of 5 groups which were divided into; negative control groups (Na-CMC), P1 (500 mg / KgBW), P2 (1000 mg / KgBW), P3 (2000 mg / KgBW) and P4 (4000 mg / KgBW). The result of histological observation showed that the exposed of variation dose of red shoot leaves extract from 500 to 4000 mg / KgBB caused any changes in liver and kidney cells. They were congestion, sinusoid dilation, mononuclear cell infiltration, and inflammation in the liver. The kidney showed appearance of necrotic cells in the proximal tubule, the infiltration of inflammatory cells, and mononuclear. The toxic effect of ethanol extract of Syzygium myrtifoliumWalp. Leaves to liver and kidney in mice.

Keywords
Histophatology, Syzygium Myrtifolium Walp, Liver, Kidney

1. Introduction

One of the plants that are currently being used as traditional medicine is the red shoot plant (Syzygium myrtifolium Walp.). This plant contains flavonoid, phenolic, and terpenoid compounds which have anti-tumor and anti-angiogenesis activities (Aisha et al., 2013); and according to Oktiadina, (2015) red shoot leaves have antioxidant activity, because they contain cyanidin-glycoside compounds. To find out the safety of its use, the toxicity test of the ethanol extract of 96% red shoot leaves has been carried out in vivo, which shows the results that the administration of red shoot leaf extract has an LD50 value of 1995 mg / KgBB and is categorized into moderate toxic; and has a toxic effect on internal organs in mice such as white spots on the lungs, blackened liver, swelling of the organs, and fluid in the abdominal cavity and thorax (Indriani, Effendi and Fadillah, 2020). Value determination The LD50 is obtained using the Thompson and Weil formula. This method was chosen because it has a fairly high level of confidence and is a method that is often used and does not require a large number of experimental animals. This method also uses a list of LD50 calculations so that the results obtained are more accurate.

In order to determine the toxic effect on tissues, further research is still needed the effect of giving ethanol extract 96% red shoot leaves to histopathological features of the liver and kidneys of mice at doses of 500, 1000, 2000, and 4000 mg / KgBW. The liver and kidneys are important organs in the body that are associated with poisoning. By conducting this research, it is hoped that the use of natural ingredients as traditional medicines in the community, especially red shoot leaves (Syzygium myrtifolium Walp.) Will be guaranteed safety, starting from therapeutic doses to doses that can cause toxic effects on liver and kidney organ tissue, especially through the liver histopathology. and kidney mice (Mus musculus).

2. Methodology

This research is a follow-up research from Indriani, Almasyhuri and Pratama, (2020), which was conducted at the FMIPA Laboratory of Pakuan University and at the Diagnostic Laboratory of the Center for Veterinary Research from March to September 2020.

2.1 Tools and Materials
2.2 Simplicia Making and Extraction

The samples used were young red leaves P1-5, red or green. 3 kg of red shoot leaves are used, collected, cleaned, and dried in an oven temperature of 50-60°C until dry. Dry simplicia is then mashed and sieved using a Mesh 30 sieve, and stored in a clean and tightly closed container (Ministry Of Health Republic of Indonesia, 1995).

The powder of simplicity was extracted with 96% ethanol solvent in a ratio of 1:10 by maceration. The container containing the sample and solvent remains conditioned at room temperature and is not exposed to direct sunlight. The soaking process is carried out for three days, stirring occasionally. The ethanol extract was filtered and the pulp was macerated again in the same way up to three times. The ethanol extract obtained is collected and concentrated. The mase rate obtained in the soaking process is then carried out by the concentration process using a vacuum dry tool.

2.3 Test of Simplicia and Extract Characteristics

a. Determination of Water Content

Determination of the water content of the simplicia powder using the Gravimetric method, namely by inserting 2 g of simplicia powder into a tared plate. Then the powder was dried at 105°C for 5 hours and weighed. After drying again and weighing every 1 hour until the difference between two consecutive weights is not more than 0.25% (Departemen Kesehatan RI, 1995).

b. Determination of Ash Content

Weighed as much as 2 g of simplicia powder and put it in a crucible that had been incandescent and tared. Annealed at a temperature of 600-700°C until the charcoal runs out, cooled and weighed.

c. Phytochemical Test

Phytochemical testing is carried out qualitatively in the form of testing alkaloids, flavonoids, triterpenoids / steroids, tannins, and saponins.

2.4 Preparation of Test Animals

Before the treatment is carried out on test animals, an ethical review is first carried out at the Animal Ethics Committee of Pakuan University to ensure that the procedure to be carried out meets the requirements of the animal ethics assessment. The tested animals to be used were 25 healthy adult male mice (Mus musculus) weighing 25 g - 30 g. Weighed the body weight and calculated CV (Coefficient of Variation) to determine the homogeneity of the experimental animals. CV values less than 15% can still be declared homogeneous (Montgomery, 1991).

The mice were grouped into 5 groups consisting of 1 negative control group and 4 treatment groups. In the dose treatment group, the rats were given a thick extract of red shoots with a dose of P1 (500mg / KgBB), P2 (1000mg / KgBB), P3 (2000mg / KgBB), P4 (4000mg / KgBB), while for negative control only a solution of Na CMC was given. 1%. Furthermore, the experimental animals were acclimatized for 7 days. Hewan test fasted before being given treatment. After being fasted, the animal is weighed and given a test preparation. The test preparation is given in a single dose of 2 mL using a sonde. The data collected in this study are the results of the control group and the treatment group. The data obtained were qualitative and quantitative data. Qualitative data were obtained by observing whether the experimental animals died in each treatment group, while quantitative data would be obtained from the counting of the number of tested animals that died.

The experimental animals that died from each treatment were then subjected to surgery to observe abnormalities in each organ and take liver and kidney organs to make histopathological preparations, which were then compared between treatments and negative controls (only 1% Na CMC was given).

2.5 Preparation of Kidney and Liver Histology

Making histopathological preparations requires the main ingredient in the form of preserved tissue. The tissue was cut and arranged in tissue cassettes, dehydrated automatically with a dehydration machine, dried with a vacuum machine, and blocked with paraffin liquid, then the blocks were cut 3-5 µm with a microtome machine and the pieces were attached to the slide. After that the slide was stained manually with hematoxylin and eosin. The staining will provide a clear balance of blue and red in the tissue, so that the cell components can be clearly identified. The histopaths from each treatment were then observed using a microscope, and compared with normal or healthy kidney and liver histopaths.
3. Result and Discussion
3.1. Result of Making Simplicia and Red Shoot Leaf Extract

The red shoot leaf simplicia powder obtained has the characteristics of a red color, distinctive aroma, bitter, and slightly septic. The final weight of the red shoot leaf simplicia powder was obtained, namely 950 g. The yield obtained from the red shoot leaves was 27.14%. The yield comes from the initial weight of fresh red shoot leaves weighing 3500 g. The yield results obtained are not much different from the research conducted by Indriani, Almasyhuri and Pratama, (2020) by 25%.

The extraction method used is maceration. The reason for choosing this method is because the substance to be drawn is flavonoid compounds that are not heat resistant, so it is suitable to use the maceration method. A total of 800 g of red shoot leaf simplicia powder was macerated with 96% ethanol solvent. The use of 96% ethanol solvent is because this solvent has the property of dissolving almost all substances, both polar, semi-polar, and non-polar (Irianty and Verawati, 2012). The ratio of simplicia powder with solvent is 1:10. The comparison is intended so that the simplicia of the red shoot leaves is completely submerged and it is hoped that it can reach the optimum extraction point. The more solvent, the greater the contact area so that the distribution of the solvent into the simplicia powder is greater and the components in the simplicia will be completely extracted (Jayanudin, Lestari and Nurbayanti, 2014). The extraction yielded 339.58 g of thick red shoot leaves.

The yield of the extract obtained in making the thick extract of red shoot leaves was 42.447%. The ethanol extract obtained was 339.58 g from 800 g of fresh simplicia powder of red leaves. The results obtained were different from (Fauzi, Ashari and Panjaitan, 2019) where the yield of thick extracts obtained was 25.80%. The difference in extract yield was due to differences in the water content of the extract or the place where the red shoots were grown, which affected the levels of secondary metabolites extracted. According Ministry Of Health Republic Of Indonesia, (2000), the yield value shows the effectiveness of the extraction process carried out. The effectiveness of the extraction process is influenced by the simplicia particle size, the type of solvent used, the extraction method, and the duration of extraction.

3.2. Moisture and Ash Content of Simplicia and Extracts

Determination of water content is important to determine the quality of the extract made because water is a medium for microorganisms to reproduce (Bartram and Pedley, 1996). The results of the determination of the water content of the red shoot leaf simplicia were 7,412%. According to Katno, (2008), the water content in the simplicia from leaves is between 5 - 9%. While the water content of the extract depends on the type of extract, the range of water content in the dry extract is <10%, 10-30% viscous extract, and liquid extract 30% (Voight, 1995). The water content obtained in the thick extract of red shoot leaves was 18.139%. Based on these requirements, the results obtained meet the requirements because they are not less than 5% and not more than 30%.

Determination of ash content aims to determine the levels of organic substances contained in simplicia. The ash content of the simplicia obtained was 3.147%. These results meet the requirements, according to (Ministry Of Health Republic Of Indonesia, 2000), the ash content of simplicia in general is less than 5%. Meanwhile, the results of the calculation of the ash content of the red shoot leaves extract was 4.006%. According to (Ministry Of Health Republic Of Indonesia, 2000). The requirement for a good extract ash content is not more than 5.9%, from the results obtained, the viscous extract ash content still meets the requirements. According to Sudarmaji, Haryon and Suhardi, (2007) the ash content of a material indicates that the presence of metal and mineral oxides in a material.

3.3 Results of Phytochemical Screening

The results of phytochemical screening of the thick extract of young leaves of red shoots showed that the extract of red shoots of red leaves contained compounds such as alkaloids, flavonoids, saponins and tannins. These results are in accordance with the results of previous studies. In the research of Haryati, Saleh and Erwin, (2015) stated that the total extract of red shoot leaves was positive for alkaloids, flavonoids and saponins. (Moerfiah, Indriani and Pramayudha, 2019), stated that the macerated and soxicated red shoot leaves contain alkaloid compounds, flavonoids, tannins and saponins. Indriani, Almasyhuri and Pratama, (2020), stated that the macerated red shoot leaves contain alkaloid compounds, flavonoids, tannins and saponins.

3.4 Maintenance of Experimental Animals

Prior to treatment, the mice to be used were grouped into 5 groups and acclimatized for 7 days. The experimental animals were grouped into negative control, P1 (500 mg / kg BW), P2 (1000 mg / kg BW), P3 (2000 mg / kg BW), and P4 (4000 mg / kg BW). Acclimatization is carried out so that the experimental animals can adapt to their new environment. Before being acclimatized, the coefficient of variation was calculated based on the body weight of the mice. The purpose of determining the coefficient of variation is to see the homogeneity of the experimental animals used. The coefficient of variation obtained was 4.081%. After acclimatization there is
an increase in body weight from 24.16 g to 30.56 g with a coefficient of variation of 10.176% and can still be declared as homogeneous because the accepted coefficient of variation is <15%.

3.5 Histopathological Examination Results

The administration of 96% ethanol extract of red shoot leaves (Syzygium myrtifolium Walp) gave a toxic effect on experimental animals by indicating the occurrence of mortality / death in experimental animals. Symptoms of toxicity are indicated by damage to organisms either due to the use of or when in an environment of toxicity or harmful effects that cause functional, biochemical, or physiological (structural) disorders that can cause pain and disrupt the body's general condition (Priyanto, 2010). The results of the observation of toxic effects were observed on liver and kidney tissue after administration of thick extract of red shoot leaves.

The histological results of the ethanol extract of 96% red shoot leaves can be seen in the table below. Based on the observations, abnormalities were found in each treatment, namely in the liver, necrosis, congestion, sinusoid dilation, mononuclear cell infiltration and inflammatory cells. The abnormalities found in the kidneys in each treatment were proximal tubular necrosis and inflammatory cell infiltration.

At the dose of P1 on the 6th day (Figure 3) the toxic substances that enter the liver are increasingly not processed properly, causing necrosis of the liver tissue. According to Kaplowitz, (2002) the target of a toxic substance in the body is the molecular structure of bile acid transport, membranes, intracellular fats, proteins and nucleic acids. As a result, the target molecule becomes a non-functioning unit and may activate secondary pathways such as apoptosis, necrosis, autophagocytes and mitochondrial disorders and other immunological reactions. Even the detoxification process of hepatic microsomal enzymes itself sometimes actually changes harmful substances to be more toxic and damages the cells themselves (Hardy, 1983).

Table 1. Result of Histology of Ethanol Extract 96% of Red Shoot Leaves

<table>
<thead>
<tr>
<th>No.</th>
<th>Organ</th>
<th>Negative control (Na-CMC)</th>
<th>P1 (500mg / Kg BB)</th>
<th>P2 (1000mg / Kg BB)</th>
<th>P3 (2000mg / KgBB)</th>
<th>P4 (4000mg / KgBB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heart</td>
<td>There are no specific abnormalities</td>
<td>Necrosis, Congestion, HE. X100</td>
<td>Necrosis (+++), Sinusoid dilatation, congestion and mononuclear cell infiltration. HE. X200</td>
<td>Necrosis (+++), Congestion, HE. X200</td>
<td>Necrosis (+++), Cell congestion and infiltration inflammation. HE. X200</td>
</tr>
<tr>
<td>2</td>
<td>Kidney</td>
<td>There are no specific abnormalities</td>
<td>Proximal tubular necrosis and Infiltration inflammatory cells. HE. X200</td>
<td>Proximal tubular necrosis. HE. X200</td>
<td>Tubular necrosis and infiltration Mononuclear cells. HE. X200</td>
<td>Proximal tubular necrosis, and cell infiltration inflammation. HE. X200</td>
</tr>
</tbody>
</table>

The liver also plays a role in detoxifying various foreign materials that may be harmful to the body. This detoxification process according to Wilkinson, (2005) is carried out through oxidation, reduction and hydrolysis (phase I reactions) or glucuronidation, sulfation, acetylation, and methylation (phase II reactions). The metabolic process of these foreign materials can sometimes disturb the balance of ions, fluids or metabolic products such as free fat or the breakdown products from the phospholipid membrane.

It is suspected that this is due to the fact that there are quite a lot of toxic substances that enter the liver, thus disrupting the digestion process of nutrients from the digestive veins. As a result, these nutrients accumulate and become toxic (mildly toxic) to the liver cells themselves (Hardy, 1983). According to Kaplowitz, (2002) damage to liver cells caused by toxic substances, generally includes the participation of metabolites against toxic substances. Furthermore, it will bring an immune response, and can even directly affect cell biochemistry. The occurrence of liver cell necrosis can be identified by changes in the cytoplasm and cell nucleus (Evans and Butler, 1993). When the plasma membrane of liver cells is damaged, various enzymes in the cytosol will be released into the blood and this can be used as a quantitative marker of the extent and type of liver cell damage (Contreras-Zentella and Hernández-Muñoz, 2016). According to (Huxtable, 1988), severe damage can inhibit the regeneration process, it can even leave a fine scar, even though the liver has returned to normal. Even in severe cases of poisoning, liver failure can cause death within 12–24 hours.
Figure 1. Histopathological Preparations of Mice Liver Organ Using HE Staining.

Information:
1. Central vein,
2. Portal tract,
3. Liver epithelial cells,
4. Congestion, and
5. Infiltration of inflammatory cells

P1. liver preparation P1 with 100x magnification; P2. liver preparation P2;
P3. liver preparations P3; P4. liver preparation P4 at 200x magnification

The histopathological features of the kidneys (Figure 4) at the dose of P1-P4 showed necrosis, and inflammatory cell infiltration. This can be caused by toxic substances that enter the kidneys, where the kidney is one of the organs that is affected by toxicity if the body is exposed to antinutrient substances (Guyton and J.E., 2007). The glomerulus and tubule are parts of the kidney that are prone to abnormalities so that it will have a morphological and functional impact if there is damage. Damage can be in the form of necrosis, cell proliferation, infiltration of inflammatory cells, the escape of large amounts of proteins, and other macromolecules, and can occur atrophy, fibrosis, edema, tubular vacuolization, congestion, and bleeding (Adinata, Sudira and Berata, 2012); (Rita Anggraini, 2008); (Suyanti, 2008).

Toxic substances that enter the body will disturb the circulatory system so that oxygen and food substances cannot be processed in the body (Price and Wilson, 2006). In this study, it was seen that there was damage in the form of glomerular edema which was marked by the presence of protein deposits in Bowman's space (Suyanti, 2008). According to Indriani, Effendi and Fadillah, (2020), edema is an increase in the volume of extracellular and extravascular fluid (interstitial fluid) which is accompanied by fluid accumulation in the tissues and serous cavities (loose connective tissue and body cavities). Edema occurs due to congestion, increased capillary permeability, and the osmotic pressure of blood and fluids, causing the escape of protein in the glomerular renal filtrate.
Figure 2. Histopathological Preparation of Mice Kidney Organs Using HE staining with 200x enlargement.

Information:
1. Proximal tubular necrosis,
2. Glomerulus,
3. Infiltration of inflammatory cells.

P1. kidney preparations P1; P2. kidney preparations P2; P3. P3 kidney preparations; P4. kidney preparations P4

When there is an increase in capillary permeability and glomerular filtration, plasma proteins and red blood cells can leak from the glomerulus so that the glomerular filtration membrane is damaged and there is swelling and edema in the Bowman's space which can cause Bowman's space to narrow (Mayori, Marusin and Tjong, 2013). This damage will interfere with the function of filtrate production and filtrate control. Glomerular enlargement (glomerulomegaly) is characterized by an increase in the volume of the glomerulus resulting in a narrowing of the Bowman space (Herlitz et al., 2010).

4. Conclusion
Pemberian ethanol extract of 96% red shoot leaves has a toxic effect on liver and kidney tissue by forming proximalis tubular necrosis, inflammatory cell infiltration, and congestion.

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References


