Histopathology of Rat Kidney Organ Due to Ethanol Extract of Phaleria Macrocarpa Treatment Induced by Isoniazid

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Abstract: The use of anti-tuberculosis drugs has side effects on human organs, especially the kidneys. Phaleria macrocarpa is now widely used as traditional medicine. The content of phaleria macrocarpa contains high antioxidants and this fruit is spread in North Sumatra. This study aims to determine the histopathological picture of induced kidney organ isoniazid (INH) by giving ethanol extract of phaleria macrocarpa. Animals test used in this study were 9 wistar rats which were divided into 3 treatment and experiments groups for 10 days. The results showed that the histopathology of kidney organ caused by INH were highly damage. However, giving Ethanol Extract of Phaleria macrocarpa at a dose of 3.4 g / 200 g was able to protect the kidneys (nephroprotector) from the toxicity of isoniazid drugs with minimal damage or almost normal. The administration of phaleria macrocarpa extract together with the anti-tuberculosis drug INH shows the protective effect of the kidneys against the INH drug.

1 INTRODUCTION

In 2030 the World Health Organization (WHO) devised a strategy to end tuberculosis (WHO, 2019). Indonesia also aims to become a tuberculosis-free country by 2050 (MOH RI, 2018). An adherence to taking anti-tuberculosis drugs plays an important role in the success of the treatment of tuberculosis (Sitorus, Fatmawati and Rahmaniah, 2017).

Development of rapid industrialization generated many negative consequence for environmental pollution affecting human health. The previous research of Nasution et all., (2015) explained that *P.macrocarpa* contained hydrogen stretching due to inter and intramolecular interaction of alcohol, phenol and carboxylic acid. This functional group will contribute to preventing metal ion from destroying organs.

The kidney is the second most common organ after the livertarget destroyer by xenobiotics. This isdue to many chemical substancesexcreted in the urine. One of the most common parts of the kidneydamage caused by chemicals is the proximal tubule. Some compounds that can be xenobiotic include alcohol / ethanol andgentamicin (Panjaitan, 2003).Microscopic changes onkidney including changes in the structure of the glomerulus, swelling or enlargementkidneys and increased numbers of fat, protein and water cells. This effect willchange the ability of the kidneys to function normally (Booggan, 2003).

The most common side effect in patients taking first-line OAT is nephrotoxicity (Pratiwi, 2018). Histologically the administration of isoniazid causes mild to moderate kidney damage (Muzika et al., 2016).

Phaleria macrocarpa fruit is one of the ingredients of traditional medicine that oftenly used to treat various diseases and has antioxidant, antimicrobial, and anti-inflammatory activity (Hendra et al., 2011). Ramadhan Research, 2019 also stated that the phaleria pacrocarpa fruit has an effect as a nephroprotector in mice which induced by paracetamol.

The results of Parapaga, Durry and Lintong, 2018 stated that antioxidant activity can inhibit and prevent oxidative damage. Antioxidants can also reduce the production of free radicals and protect body cells from oxidative stress (Octaria, 2019).

According to Yatman (2012), the antioxidant process throughoxidation and reduction reactions that form oxidizing free radicalswith reactive oxygen. Because of its reactivity, free radicals willoxidizes beneficial substances for the body causing a numberdamaged body tissue. Hence it is easily

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oxidized, free radicals, for that matterperoxyl radical (ROO) will oxidize xanthone rapidly so that it is radicalthe peroxyl will turn into R-H. This change occurs because of the oxygen moleculereduced by garsinone B as a xanthone derivative, the reaction is inhibitoryfree radicals of various types.Free radicals can interfere with cellular function by performinglipid peroxidation resulting in damage to cell membranes. This damage can becauses changes in electric charge in cells, changes in osmotic pressure,causes cell swelling and ends in cell death.

Nakatni et al., (2004) conducted a study on the in vitro anti-inflammatory activity of γ-mangostin against the synthesis of PGE2 and cyclooxygenase (COX) in glioma cells mouse C6. These two compounds and enzymes are the most important mediators in the inflammatory reaction. From this research results can be made:mangostindirect PGE2 production in the inflammatory process.

In order to develop research on extracts of the crown of the godsas a herbal medicine, this research was conducted with the aim to find outthe effect of giving the extract of the Dewa's Crown on the histopathological picture of the kidney of rats which was induced by isoniazid and the optimal dose of the extract had an effect on the liver and kidneys. The results obtained are expectedused as a source of information about the benefits of extract of the crown of gods especiallyon the kidneys and serve as the basis for further researchdevelopment of mangosteen peel extract as a standardized herbal medicine.

This study aims to determine the histopathological picture of kidney organ induced by INH (Isoniazid) by giving ethanol extract of Phaleria macrocarpa fruit.

Nasution et al., (2019) Cd(II) ion is a heavy metal that has atoxic abilty in the human body. phaleria macrocarpa has been used as anticancer, Diabetes Mellitus and antimicrobe because its consists of flavonoid, steroid and tannin. The result of exsperimental rats exposed witg Cd(II) ion, there are significant decreasing of all the observed parameters including MAD, SGOT and SGPT with percentage 71,5 %, 72,1 % and 93, 6 % respectively. The rats given with the antidote of phaleria macrocarpa flesh fruit were able to protect the liver from damage due to exposure to Cd(II) as seen from the decrease in liver function enzyme parameters namely SGOT and SGPT

2 METHOD

This research (conducted at the Pharmacy Laboratory, University of North Sumatra in December 2019) used an experimental design with a post-test only control group design pattern. Experimental samples were 9 male Wistar white rats (Rattus Novergicus), weight 150-200 grams were used. Rats were in accordance with the code of conduct issued by the Ethics Committee of the Faculty of Medicine, University of Prima Indonesia.

The tools used were laboratory glass, analytical balance, blender, incubator, oven, rotary evaporator, water bath, animal scales, animal house, injection equipment, surgical instruments, microtome, object glass, cover glass, light microscope. The ingredients used were phaleria macrocarpa, aquadest, isoniazid, CMC-Na 0.5%, 10% formalin buffer, 96% ethanol, preparations dyes.

The phaleria macrocarpa fruit was separated from the skin and seeds and washed with running water. The phaleria macrocarpa meat was cut into small pieces and then dried and blended until smooth into powder, then put into a container that can be tightly closed.

The 500 mg simplicia powder was macerated with 96% ethanol, then evaporated with a rotary evaporator at ≤70oC. Re-evaporated with waterbath (water bath) until the extract results become thick (Lestari, 2019; Ramadan, 2019).

Nephroprotector Effectiveness Test Samples were divided into 3 groups, so that each group amounted to 3 rats. The treatment of each group was as follows: Control group (P1) without treatment (only aquades). Treatment Group 2 (P2) isoniazid 300 mg / 70 kg BW induced. Treatment group 3 (P3) was given ethanol extract of Crown Fruit of a dose of 3.4 g / 200 g BW with isoniazid induction dose of 300 mg / 70 kg BW. The treatments were orally contucted once per day for 10 days. At the end of the experiment, the rats were anesthetized with chloroform before being dissected, kidney organs were taken and then put into containers containing 10% formalin buffer that had been labeled. Preparations made with a thickness of 4-6 mm, stained with HE and viewed under a microscope to see histopathological changes.

3 RESULTS

3.1 Histopathology of the Kidney Organs

The results of histopathology of the renal organs examined in the control group are given in Figure 1. In the control rat the glomerulus and renal tubules were seen in normal condition, the nucleus was clearly visible and the renal capsule was also clearly

visible. Mice induced with isoniazid 300 mg / 70 kg bw (KP2) were found with moderate glomerular atrophy with necrosis. We found damage in the kidneys as shown in Figure 2.

Mice given Ethanol Extract of Phaleria Macrocarpa (EEPM) with isoniazid induction (KP3) were seen with mild glomerular atrophy with necrosis. In contrast to those who were only given INH only in group 2, there was a lot of damage and necrosis in the kidney organs. The administration of the extract phaleria macrocarpa fruit can proverly protect the kidneys from isoniazid induction.

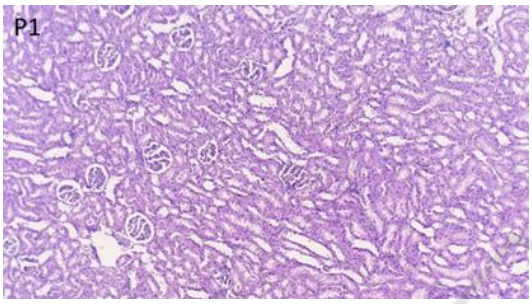

Figure 1. Histopathology of rat kidney organ controls (P1). 10 x 10 magnification

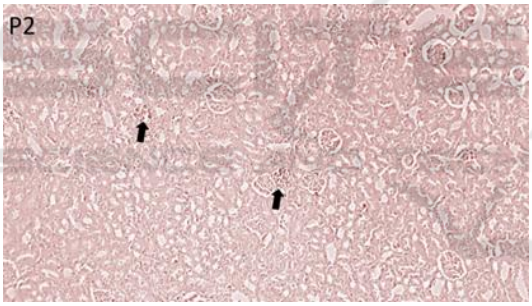

Figure 2. Histopathology of rats kidney organ (P2) = INH 300 mg / 70 kg bw.

Description: glomerular atrophy (black arrow).

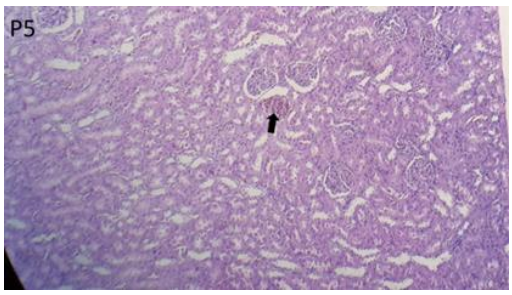

Figure 3. Histopathology of rats kidney organ (P3) = EEPM + INH. Description: glomerular atrophy (black arrow).

According to Zakhari et al., (2006), the results of alcohol metabolism that occur inliver, namely the formation of acetate, acetyldehyde, and increased reactive oxygenspecies (ROS) enter the systemic blood circulation which can damagethe structure of the cells of the extra hepatic tissue namely the brain, lung, heart and tissuekidney. ROS which are highly reactive can cause the breakdown of molecular complexescellular (Wu and Caderbaum, 2005). After ethanol intoxication,the balance between prooxidants and antioxidants is disturbed thuscauses oxidative stress from biomolecules, such as fat, protein, or DNA,and ultimately cause cell damage (Das and Vasudeven, 2007).

According to Yatman (2012), the process of antioxidants through oxidation reactions andreduction which forms free radicals which are oxidizing agents withoxygenreactive. Because of their reactivity, these free radicals willoxidize substances that arebeneficial to thebody, causing a number of body tissues to be damaged. Bybecause it is easily oxidized, free radicals, in this case theperoxyl radical (ROO) willoxidizes xantones quickly, so that the peroxyl radical will changeto be R-H. This changeoccurs because the oxygen molecule is reduced by garsinon Bas a xanthone derivative, its reaction can inhibit free radicals of varioustype.

The molecular structure of each of the cell and tissue flavonoid groupsthe body is constantly exposed to the damaging effects caused by free radicalsand reactive oxygen species (ROS) free radicalsnormally formed during oxygen metabolism or induced by damageexogenous. Free radicals can interfere with cellular function by performinglipid peroxidation resulting in damage to cell membranes. This damage can becauses changes in electric charge in cells, changes in osmotic pressure,causes cell swelling and ends in cell death. Flavonoidscan interfere with more than 3 different free radical generating systems, andcan also enhance endogenous antioxidant function. Antioxidant activityhere is the antioxidant mechanism of flavonoids, namely binding radicalsdirectly (direct radical scanning venging), through nitric oxide, xanthinoxidase, immobilization of leukocytes, interactions with other enzyme systems (Nijveldtet al., 2001).

4 RESULTS

This research was conducted to determine the effectiveness of etal extract of Phaleria macrocarpa fruit on nephroprotectors in isoniazid-induced white rats. Treated the rats with the EEPM at a dose of 3.4 g / 200 g bw can neutralize kidney damage due to isoniazid with kidney histology indicators in mice.

Based on the results of research in the negative control group (mice without treatment that was only given aquadest) had a slight inflammation. Supposedly in the negative control group there was no damage to hepatocyte cells, this might be caused by external variables that could not be controlled, such as the psychological condition of the rat and the

initial condition of the liver and kidney of the rat before treatment. Changes in the environment of mice also affect the behavior patterns of mice (Suharyadi, Sukohar and Muhartono, 2014).

The histopathological picture assessed in the kidney organ in the form of glomerular atrophy is characterized by a decrease in the size of the glomerular tissue due to the reduced number of cells. The widening of the tubular lumen was due to the presence of a protein cast or protein deposition on the tubules.

According to Anggriani(2008), microscopic picture of epithelial cells of the proximal tubuleswell with granular cytoplasm due to shifting of waterextracellular into cells. This shift occurs because the toxin causeschanges in the surface electric charge of tubular epithelial cells, active transport of ions and acidsorganic, and the ultimate concentrating ability of the kidneycauses the tubule to be damaged, the flow decreases. Picture of swelling of these cellscalled albuminous degeneration or parenchymal or cloudy degenerationswelling (cloudy swelling). This may cause the tubular lumenproximal narrowing to close. According to researchconducted by Liu et al., (2002) who stated that giving ethanol50% with a dose of 10 ml / kg of rats can cause kidney damage.

The treatments with the Phaleria macrocarpa extract for 10 days did not cause any toxicity, because there were no deaths and poisoning in white rats during the study. Based on the histopathology that has been studied, the EEPM is able to protect the kidneys due to isoniazid induction because it has high antioxidants.

The antioxidant content of flavonoids as inhibitors of CYP3A activity and acts as a free radical scavenger (Hassanin, Abd El-Kawi and Hashem, 2013). Flavonoid compounds contained in the phaleria macrocarpa fruit have an influence in protecting the kidneys.

5 CONCLUSIONS

The results showed that an effect after giving an EEPM at a dose of 3.4 g / 200 g bw on the kidney tissue damage were highly reduced as evidenced by histopathological examination. Preventive use of the EEPM can act as a protective kidney when consuming OAT INH together.

6 SUGGESTION

The next researcher is suggested to design experiment using the phaleria macrocarpa fruit in the different dosage of concentration with a longer trial period.

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