



Expression of Kidney White Rats Diabetes Mellitus after Therapy of Sinom Ethyl Acetate Fraction Mixed Lime and Honey

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ABSTRACT

This study aims to prove that the administration of the ethyl acetate fraction sinom mixture of lime and honey (SCJM) can improve the histological structure of the kidney of Sprague Dawley rats (SD) diabetic were divided into six groups with four replications each. The K1 group as a positive control was not given a sample, the K2 group was given a sample dose of 50 mg/kg BW, the K3 group was given a sample dose of 100 mg/kg BW, and the K4 group was given a sample dose of 150 mg/kg BW, the K5 group was given an extract dose of 200 mg/kg BW, and positive control group K6 which was carried out for 21 days. The rats were then sacrificed and the kidney organs were taken for histopathological preparation with Hematoxylin Eosin (HE) staining. The results showed that there was fatty degeneration and necrosis of the kidneys. Kruskal Wallis test results from all treatments showed $p = 0.001$ for both fatty degeneration and necrosis ($p < 0.05$)

INTRODUCTION

Background

Diabetes mellitus (DM) or commonly referred to as diabetes is a chronic metabolic disorder disease (Ministry of Health, 2014). People with diabetes in 2015, there were 415 million adults in the world, an increase of 4 times compared to 1980, which was 108 million. The prevalence of diabetes in Southeast Asia reached 8.6% in 2014 (WHO, 2018). Indonesia ranks seventh in the world for the highest prevalence of diabetes with an estimated 10 million people in 2015 and reaching 6.9% in 2016 (Risksedas, 2016; WHO, 2018)

Diabetes mellitus (DM) is a serious common metabolic disorder and is associated with many functional and structural complications (Gispén and Biessels, 2000). Diabetes mellitus is characterized by increased blood sugar levels or hyperglycemia. This condition results from defects in insulin secretion, insulin action, or both.

Chronic hyperglycemia refers to continued damage to organs and dysfunction or failure of multiple organs. Damage caused by diabetes mellitus is known as microangiopathy or small blood vessel damage which includes nephropathy, neuropathy, and retinopathy (Nelson, 2010)

LITERATURE REVIEW

Sinom mixture of lime and honey (SCJM) is classified as a plant that acts as a functional food and has been tested to contain a lot of nutritional value. Wiradnyani, N., K (2014) stated that the antioxidant content of SCJM varies depending on the results of the resulting fractions including turmerone, ar-turmerone, riboflavin, 9-octa monounsaturated fatty acid decanoic fatty acid, ascorbic acid, and has antidiabetic and anticancer biological compounds. The water fraction of SCJM is able to repair pancreatic damage directly by increasing glucose uptake by tissues, preventing gluconeogenesis in the liver, or absorbing glucose into muscle and adipose tissue (Jaiswal et al., 2009)

Recent studies have concluded that increased plasma glucose results in damage to pancreatic cells. SCJM which is a source of ascorbic acid is able to stimulate insulin release so that it is used as a hypoglycemic agent (Wiradnyani, 2019). Research by El-Desouki et al. (2015) who tested the effect of high-dose Moringa leaf extract (400 mg/kg body weight) on diabetic rats for 30 days showed that Moringa was able to reduce blood glucose levels, increase insulin production, and restore the work activity of pancreatic cells

A study by Ndong et al. (2007) and Al-Malki and El-Rabey (2015) demonstrated the effectiveness of using Moringa seed extract in restoring histologically damaged kidneys and pancreas. These properties refer to the antioxidant content of Moringa in the form of glucomoringins, phenols, and flavonoids. Paliwal et al. (2011) stated that the antioxidant content of Moringa oleifera has nephroprotective activity in mice undergoing renal carcinogenesis. However, research on the administration of the SCJM Ethyl Acetate fraction to the renal histopathological features of SD White rats has never been done.

High glucose levels are the main cause of structural changes in the kidneys. Mesangial cells produce TGF-1 under conditions of hyperglycemia, resulting in increased glucose consumption and transport due to overexpression of GLUT-1 mRNA and protein. This condition causes metabolic abnormalities in mesangial cells. Impaired kidney function in DM patients is indicated by an increase in serum creatinine, uric acid, and blood urea nitrogen (Francesco and Loreto, 2005).

Based on the description above, a problem can be formulated, namely whether the administration of SCJM causes kidney histopathology improvements in SD white rats.

METHODOLOGY

Research

This study used a mixture of lime and honey which was purchased from the Denpasar local market, 25 white rats Sprague Dawley obtained from Viterinerien Surabaya as experimental animals-, and streptozotocin as a substance for making diabetic rats.

Preparation and Treatment

This study used 24 male Sprague Dawley (SD) rats aged 3-4 months with a body weight of about 150-200 grams which were first adapted to the cage environment for a week. SD rats were given a single dose of streptozotocin (STZ) of 45 mg/kg BW intraperitoneally. On the third day after STZ injection, all SD rats were measured for blood glucose levels so that they met the DM criteria, namely 400-500 mg/dl. SD Diabetes Mellitus (DM) rats were then grouped into six groups. Group I (without DM positive control ethyl acetate fraction). Group II (control diabetes, SCJM ethyl acetate fraction at 0 mg/kg BW/day), group III (SCJM ethyl acetate fraction at 50 mg/kg BW/day), group IV (SCJM ethyl acetate fraction at 100 mg/kg BW /day), group V (SCJM ethyl acetate fraction 150 mg/kg BW /day), group VI (SCJM ethyl acetate fraction 200 mg/kg BW/day), On the fourth day after STZ injection, SD DM white rats began to be treated using SCJM ethyl acetate fraction with doses according to the treatment provisions of each group. The SCJM ethyl acetate fraction was administered orally using a gastric probe and administered for 21 days. On the fourth day after STZ injection, SD DM rats began to be treated using SCJM ethyl acetate fraction with doses according to the treatment provisions of each group. The SCJM ethyl acetate fraction was administered orally using a gastric probe and administered for 21 days. After 21 days of administration of SCJM ethyl acetate fraction, SD DM rats were sacrificed and their kidneys removed. These organs were stored in Neutral Buffered Formalin for later processing into histopathological preparations.

Preparation of SCJM Ethyl Acetate Fraction

The preparation and manufacture of the Sinom ethyl acetate fraction of a mixture of lime and honey is done by: adding 100 ml of Sinom drink a mixture of lime and honey into a separatory flask that has been dried in an oven for 15 minutes at 100°C, then adding 100°C of ethyl acetate solvent 100 ml, shaken 10

times and allowed to stand for 30 minutes. The resulting fraction was then evaporated at a temperature of 45°C and a pressure of 280 bar to separate the solvent. The results of the ethyl acetate fraction of a mixture of lime and honey are ready to be given to white rats with diabetes mellitus (Wiradnyani, 2018b). All crude ethyl acetate fractions were stored in a refrigerator at 10°C before being analyzed and applied to experimental animals.

Histopathological Preparation

The procedure for making histopathological preparations refers to the method reported by Muntiha (2001). The first step is to fix the kidneys using Neutral Buffered Formalin 10% for 48 hours. The organs were then cut to a size of 1 x 1 x 1 cm and inserted into a tissue cassette. Tissue sections were dehydrated successively using 70%, 80%, 90% alcohol, absolute alcohol I, and absolute alcohol II for two hours at each alcohol concentration. The next step is clearing or the process of removing alcohol from the tissue by soaking the tissue in xylene compounds. After the clearing process, the network must go through the embedding process or impregnation to remove the clearing agent from the tissue. In the embedding process, the tissue is infiltrated by paraffin compounds so that the initially soft tissue becomes hard and easy to cut using a microtome. The tissue then went through a blocking process so that the tissue was printed in paraffin blocks and stored in the refrigerator for 24 hours. The paraffin blocks were then cut using a microtome with a thickness of 4-5 microns. The cutting results were floated in warm water at 60°C to avoid the formation of folds. The preparation was then removed and placed on an object glass for Harris Hematoxylin-Eosin staining

Histopathological Examination

Observation of kidney histopathological preparations was carried out under a binocular microscope with 400x magnification. Observations were made in five fields of view on the various changes that occurred, then scored based on the average tissue change. The criteria for changes observed were fatty degeneration and necrosis of kidney tissue with a score of 0 for no fatty degeneration/necrosis, a score of 1 for fatty degeneration/focal necrosis, a score of 2 for fatty degeneration/multifocal necrosis, a score of 3 for fatty degeneration/diffuse necrosis.

Statistical Analysis

This study used a completely randomized design (CRD) consisting of six treatment groups and four replications each. The data were analyzed using the Kruskal-Wallis non-parametric statistical test, if it had a significant effect, it would be continued with the Mann-Whitney test. Both tests were operated using SPSS version 17.

RESULTS

Outcome

It is found that the Kruskal Wallis test analysis of all treatments showed $p = 0.001$ for both fatty degeneration and necrosis ($p < 0.05$). This indicates that the administration of the SCJM ethyl acetate fraction has a significant effect on the improvement of the histological structure of the kidney in the form of a decrease in the amount of fatty degeneration and necrosis. Because the results of the analysis obtained have a significant effect, then proceed with the Mann-Whitney test to see the differences between treatments

The results of the Mann-Whitney test for fatty degeneration showed that there was no significant difference between K1 (control group) and K2 (ethyl acetate fraction SCJM at a dose of 50 mg/kg BW) ($p > 0.05$), while between K1 and K3 (ethyl acetate fraction) SCJM acetate at a dose of 100 mg/kg BW), K4 (ethyl acetate fraction SCJM at a dose of 150 mg/kg BW), K5 (a SCJM ethyl acetate fraction at a dose of 200 mg/kg BW), showed a significant difference ($p < 0.05$).

The results of the Mann-Whitney test between K2 and K3 showed that there was no significant difference ($p > 0.05$), whereas if you looked at the comparison of results between K2 and K4, K5, and K6 treatments, there was a significant difference ($p < 0.05$). The results which showed that there was a significant difference ($p < 0.05$) were also obtained from comparing K3 with K4, K5, and K6. The comparison between K4 and K5 showed that there was a significant difference ($p < 0.05$), on the other hand, there was no significant difference between K4 and K6 ($p > 0.05$). The results of the comparison between K5 and K6 showed that there was a significant difference ($p < 0.05$). The results of the Mann-Whitney test for necrosis between treatment groups K1 and K2, K3, and K6 showed no significant difference ($p > 0.05$), while between K1 and K4 and K5 showed a significant difference ($p < 0.05$). Comparison of the amount of necrosis between K2 and K3, K4, K5, and K6 showed a significant difference ($p < 0.05$).

The K3 group showed necrosis with a significantly different score ($p < 0.05$) from the K4 and K5 treatment groups, but when compared with K6, the test showed no significant difference ($p > 0.05$). Necrosis scoring results between K4 with K5 and K6 resulted in a significant difference ($p < 0.05$). Significantly different results ($p < 0.05$) were also obtained by comparing the amount of necrosis between K5 and K6.

The results of microscopic observations showed that the glomerulus and tubules experienced fatty degeneration and necrosis. The damage was found in all treatment groups. In fatty degeneration, the tubular epithelial cell nuclei are pushed to the edge and fat vacuoles are seen filling the cytoplasm. Necrosis that occurs in the tubules includes epithelial loss, detachment of the basement membrane, pyknotic cell nuclei, ruptured cell nuclei (karyorrhexis), and missing cell nuclei (karyolysis), to desquamation or loss of a collection of epithelial cells due to no surrounding tissue that holds them.

The K2 treatment group (diabetes mellitus without SCJM ethyl acetate fraction) showed fatty degeneration and necrosis of the tubules and glomeruli. Most of the tubules undergo necrosis and fatty degeneration at the same site. The K3

treatment group (administration of the SCJM ethyl acetate fraction at a dose of 50 mg/kg BW) had a microscopic picture that was not much different from the K2 treatment group. Kidney organs in the K4 treatment group (administration of ethyl acetate fraction SCJM at a dose of 100 mg/kg BW) began to show improvement. The Tubular structure was observed to be more normal than the K3 group with less fatty degeneration and necrosis.

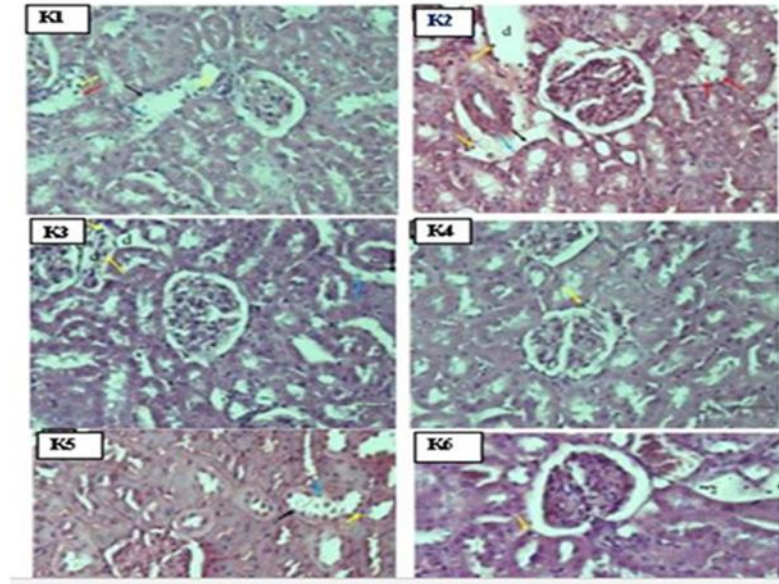


Figure 1. Image of the Histopathological Overview of the Kidney After

Figure 1: Image of the Histopathological overview of the kidney after administration of various doses of SCJM ethyl acetate fraction. Information: K1 = Group 1 (positive control DM), K2 = Group 2 (SCJM ethyl acetate fraction dose 0 mg/kg BW), K3 = Group 3 (SCJM ethyl acetate fraction 50 mg/kg BW), K4 = Group 4 (Fraction SCJM ethyl acetate at 100 mg/kg BW, K5= Group 5 (SCJM ethyl acetate fraction at 150 mg/kg BW), K6= Group 6

Kidney damage in the K5 treatment group (ethyl acetate fraction SCJM dose 150mg/kg BW) was reduced in the five visual fields. The tubular structure appears to have improved to near normal with the epithelium still attached to the basement membrane. Treatment group K6 (ethyl acetate fraction SCJM dose 200 mg/kg BW) showed better organ structure improvement than before. The Cortical structure is close to normal.

DISCUSSION

Necrosis is focal, whereas fatty degeneration is often absent. These results are in agreement with the SCJM ethyl acetate fraction. Treatment group K6 (ethyl acetate fraction SCJM 200 mg/kg BW) showed worse results than K5. These results indicate that the dose of 200 mg/kg BW is prooxide to the rat kidney, causing more lesions than the dose of 150 mg/kg BW and 100 mg/kg BW, but the dose of 200 mg/kg BW is better than the dose of 150 mg/kg BW and the control group (0 mg/kg BW). The SCJM ethyl acetate fraction at a dose of 200 mg/kg BW caused less fatty degeneration than the 150 mg/kg BW dose-, but produced more necrosis than the 100 mg/kg BW dose

The proximal tubule is the part of the nephron that is most easily injured due to toxic substances or ischemia-, because in the proximal tubule absorption and secretion processes occur so that toxic substances are concentrated higher. Other causes of proximal tubular damage include high levels of cytochrome P-450 which can activate toxic substances (Mardiastuti,2002). The process of renal tubular damage begins with the entry of toxic substances into the tubular epithelial cells which are then responded to in the form of degeneration. Fatty degeneration indicates the occurrence of nephritis due to toxic substances in the tubules. Further epithelial damage can be in the form of cell necrosis and desquamation. The desquamated tubular tissue then turns into connective tissue and causes a decrease in kidney function. A decrease in kidney function of more than 25% can cause kidney failure (Mardiastuti,2002)

In the case of diabetes mellitus, the state of angiopathy causes narrowing and blockage of blood vessels, including blood vessels leading to the kidneys. Blood plasma of diabetics has high viscosity (viscosity), so blood flow becomes slow Blood plasma of diabetes has high viscosity (viscosity), so blood flow becomes slow necrosis or cell death of various organs including the kidneys (Wibowo, 2004). Focal necrosis can be replaced by healthy cells through the process of cell regeneration if the cause is removed, whereas in the case of diffuse necrosis, dead cells will be replaced by connective tissue (cirrhosis) (Mardiastuti,2002).

CONCLUSIONS AND RECOMMENDATIONS

This study is the administration of SCJM ethyl acetate fraction could improve renal histopathology of experimental diabetes mellitus SD white rats by decreasing the amount of fatty degeneration and necrosis. The doses of SCJM ethyl acetate fraction that can repair kidney damage are 50 mg/kg BW, 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW. A dose of 150 mg/kg BW has the best effect on repairing kidney damage

FURTHER STUDY

This research will be continued by conducting clinical trials, namely the sinom fraction of ethyl acetate, a mixture of lime and honey, which will be tested on humans suffering from diabetes mellitus.

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