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Histopathological Study of Streptozotocin-Induced Liver Regeneration of Wistar Rats After Administration of Pumpkin Leaf Ethanol Extract

Niluh Puspita Dewi*, Windi Olivia Kawanga, Debora Rislianti Lakiu
Department of Pharmacy, STIFA Pelita Mas Palu, Central Sulawesi, Indonesia
*Corresponding author e-mail: niluhpuspitadewi978@gmail.com

ABSTRACT

Traditional medicine has been known starting from hereditary information, then its efficacy is confirmed by scientific research results. Plants that have been studied have an influence in reducing levels of hyperglycemia, creatinine and kidney urea, one of which is the pumpkin plant which is believed to be derived from wild plants in Ambon and is easy to obtain and many benefits can be obtained from consuming these plants. This study aims to prove that the ethanol extract of pumpkin leaf can improve liver histological structure of Wistar rats streptozotocin and find out the effective dose in regenerating the liver cells of wistar rats. Wistar rats were divided into six groups, so that each group has five replications. The control group was not given the extract, and the treatment group was administered the extract with three dose of 125 mg/kg bw, 150 mg/kg bw, and 175 mg/kg bw which were carried out for three weeks. Rats were then sacrificed and the liver organs were taken for the histopathology preparations with Hematoxylin-Eosin staining (HE). The results showed fatty degeneration and necrosis of the livers. The results of the Mann-Whitney test analysis showed that there was a significant difference in liver histopathology scoring from each treatment group. The Ethanol extract of pumpkin leaves at dose of 150 mg/kg bw gave an effect on the regeneration of liver cells of wistar rats with a damage score of 1.4 and had no effective dose.

***Keywords**: Histopathology, Hematoxylin-Eosin, regeneration of liver cells.*

INTRODUCTION

The Indonesian people have long known and used traditional medicine as a prevention, treatment, and increase endurance. Knowledge of medicinal plants is based on experience and skills that have been passed down from generation to generation by our ancestors. The use of traditional medicine by the community as an alternative treatment for oneself. Traditional medicine has been known starting from hereditary information, then its efficacy is confirmed by scientific research results (Tandi, et al.., 2018).

Plants that have been studied have an influence in reducing levels of hyperglycemia, creatinine and kidney urea, one of which is the pumpkin plant (*Cucurbita moschata* Durch) which is believed to be derived from wild plants in Ambon and is easy to obtain and many benefits can be obtained from consuming these plants. The phytochemical content in pumpkin produces active compounds of flavonoids, saponins, phenolics, terpenoids, protein, calcium, vitamins A, B, C and E, so it is trusted by the public as antidiabetic (Suwanto, et al., 2019).

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both (Perkeni, 2015). Diabetes mellitus is a condition in which the concentration of glucose in the blood is chronically higher than normal (hyperglycemia) due to insulin deficiency or ineffective insulin function. This disease is known as a disease resulting from a modern lifestyle (Soegondo & Soeyono, 2018).

Previous research on the antidiabetic effects of pumpkin flesh and seeds extracts in streptozotocin induced mice showed that the ethanol extract of pumpkin flesh and seeds at a dose of 150 mg/kg BW showed a significant decrease in blood glucose (Novarianti, et al., 2018). Another study on the effect of Pumpkin Seed Extract on Glucose, Cholesterol and Histopathological Appearance of Rat Pancreas showed that pumpkin seed extract doses of 360 and 450 mg/kg bw were effective in reducing pancreatic tissue degeneration in diabetic hypercholesterolemic rats. (Tandi, et al., 2018).

Histology is the science that describes the structure of specific animals and the relationship between cell and tissue structure and function. Histopathology is the branch of biology that studies the condition and function of tissues in relation to disease. Histopathology is very important in relation to the diagnosis of disease because one of the considerations in the diagnosis is through the observation of suspected disturbed tissue. Analysis of the histological condition of the organ/tissue can be seen from changes in morphology, structure and indi cations of damage/infection/mutation due to disease, toxins or other mutagenesis (Suwanto & Rahmawati, R, 2019).

Liver is the main organ in the process of drug biotransformation so that the toxic effects of drugs can occur in these organs. Liver damage due to toxic substances can be influenced by several factors, namely the type of chemical involved, the amount of the dose given, and the length of exposure to the substance (Handani, et al., 2018). Based on this, the researchers wanted to observe whether there was a change in the histopathological picture of the liver of wistar rats induced by streptozotocin after being given ethanol extract of pumpkin leaves.

MATERIAL AND METHODS

Chemicals and Instrument

The tools used are surgical instruments, glassware (pyrex ®), 40 mesh sieve, maceration vessel, embedding center, Floatation bath, scissors, glucometer (Accu-Chek ®), test animal cages, basket tissue processor, rotary microtome, Olympus CX 21 microscope plus light camera, mortar and stamper, waterbath, microtome knife sharpener, microtome knife, tweezers, dropper, Rotary vacuum evaporator (Heidolph), Slide staining racks, 3 ml oral

sonde (One Med Health Care), 3 ml injection syringe (One Med Health Care), spot plates, test tube (pyrex ®), and analytical balance (Ohaus).

The materials used are distilled water (aqua), citrate-buffer saline (citric acid and sodium citrate), ethanol 96% (Merck), 10% PBS formalin, chloroform, pumpkin leaf, dragendrof LP, glibenclamide, Liebermann-Burchard, solution Mayer Hematoxylin-Eosin, 0.5% Na CMC, sodium hydroxide (Merck), sodium chloride (PT. Widatra Bhakti), streptozotocin (Bioworld USA), magnesium powder (Merck), paraffin, and standard feed.

Extraction

Pumpkin leaf extract was made by maceration method using 96% ethanol as solvent. The simplicia powder was weighed as much as 1000 grams and then put into a maceration vessel using 6 L of ethanol solvent, closed and left for 3x24 hours protected from light while stirring occasionally. The vessels used are 3 maceration vessels. The maceration results were filtered using filter paper and the filtrate was obtained, then evaporated or separated the solution using a Rotary Vacuum Evaporator at a temperature of 60^{o}C and continued with thickening carried out using a water bath at a temperature of 60^{o}C until a thick extract was obtained (Tandi, et al.., 2018).

Phytochemical Screening Test

Preliminary tests are used to detect the chemical composition of plants based on their groups as initial information to determine the class of chemical compounds that have biological activity from a plant among others alkaloids, flavonoids, saponins and tannins (Tandi, et al.., 2018).

Test Materials Manufacturing

The ethanol extract of pumpkin leaves was weighed to make a test suspension with 0.8 grams (100 mg/kg bw), 1.6 grams (200 mg/kg bw) and 3.2 grams (400 mg/kg bw) respectively. Subsequently, 0.5% Na CMC was added to each extract and the volume was made up to 100 ml with distilled water and then shaken until homogeneous (Tandi, et al.., 2018).

Preparation of Streptozotocin

Streptozotocin was weighed as much as 0.32 grams and then dissolved using citrate-buffer saline with a pH of 4.5 to 100 ml, then induced in rats via intraperitoneal (ip). The dose of streptozotocin is 40 mg/kg bw (Tandi, et al.., 2018).

Antidiabetic Effect Test

The study was conducted using test animals in the form of 30 male white rats of the wistar strain. The test animals used were approved by the Animal Ethics Commission of the Medical and Health Research Ethics Committee of Tadulako University. In testing the effect of ethanol extract of pumpkin leaves on the histopathological study of the liver of male wistar rats induced by streptozotocin. 30 male white rats were

divided into 6 groups and adapted for 2 weeks in the laboratory and given standard feed. On day 0 after adaptation, the rats were fasted for 16 hours, then the initial blood glucose levels were measured. After measuring initial blood glucose levels, on the same day, rats were induced by streptozotocin with 40 mg/kg BW intraperitoneally. The seventh day after induction, the rats were fasted for 16 hours and then re-measured the blood glucose levels of the rats after induction. After the fasting blood glucose levels of the rats had reached a hyperglycemic state (>200 mg/dL), they were given oral treatment for 21 days. The measurement data of blood glucose levels before and after the treatment obtained were recorded and analyzed.

Liver Histology Test

Test animals were killed by neck dislocation. The dead animal is placed on a fixation board with the stomach pointing up. The cuts were made on the skin of the abdomen crosswise until the organs inside the rat's stomach were visible. The rat liver was then taken and stored in a special container containing 10% formalin.

Microscopic examination is carried out under a microscope to see the morphological changes of the specimen being examined. The examination was carried out 5 times in the field of view and then the average score of abnormalities obtained or the percentage of damage in the 5x field of view was averaged (Tandi, et al.., 2018).

RESULTS AND DISCUSSION

The ethanol extract of pumpkin leaves was obtained through the extraction process with the maceration method, the ethanolic extract of pumpkin leaves obtained was 60 grams with a percentage yield of 9%. Based on the results of the phytochemical screening test, pumpkin leaf extract contains secondary metabolites, namely alkaloids, flavonoids, and saponins.

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The results of observations of histopathological preparations of the liver of wistar rats induced by streptozotocin at a dose of 40 mg/kg bw and administration of various doses of ethanol extract of pumpkin leaves at 125, 150 and 175 mg/kg bw were performed using an Olympus Cx-21 microscope with 400x magnification. From the scoring data on the level of liver damage of wistar rats, the average level of damage was obtained, namely normal control (0), negative control (2.4), positive control (1.6), treatment group with a dose of 125 mg/kg bw (2.4) , the treatment group at a dose of 150

mg/kg bw (1,4) and the treatment group at a

dose of 175 mg/kg bw (2).

Table 1. Wistar rat liver histopathology score score

Treatment Group	Test Animal Liver Damage Score					Average±SD
	1	2	3	4	5	
Normal control	0	0	0	0	0	0±0[a]
Negative control	3	2	2	2	3	2,4±0,54
Positive control	2	1	1	2	2	1,6±0,54
Dose of 125 mg/kg BW	3	2	3	2	2	2,4±0,54
Dose of 150 mg/kg BW	2	1	2	1	1	1,4±0,54
Dose of 175 mg/kg BW	3	2	2	2	1	2±0,70

Description of liver/liver damage score : Score 0 : No damage / normal (0)
Score 1: light damage (<25%)
Score 2: moderate damage (25%-50%)
Score 3: heavy damage (>50%)

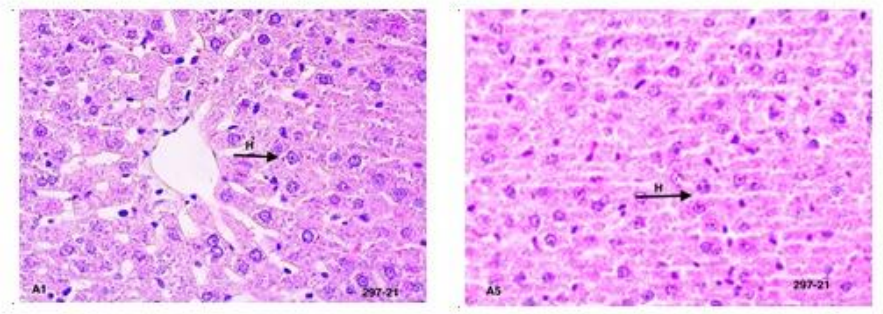

Figure 1. Histopathology of the liver of male white rats 400X magnification with H&E staining score 0 (normal); Arrows marks, indicated no changes. Normal in hepatocytes.

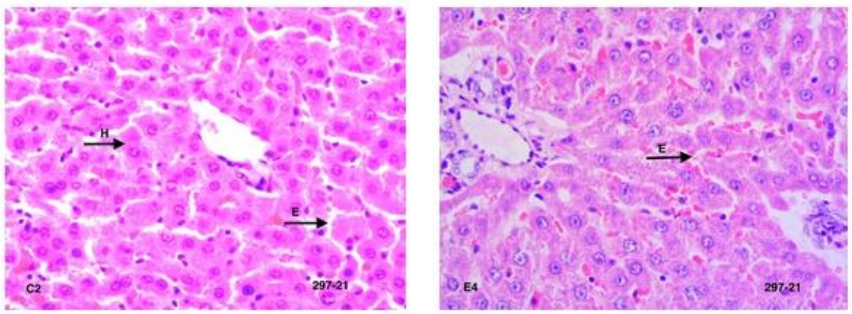

Figure 2. Histopathology of the liver of male white rats magnified 400X with H&E staining score 1 (mild damage)
Arrows marks, indicated :
minor damage. Necrotic cells > 25%. E (edema), H (hepatoside) normal
minor damage. Necrotic cells > 25%. E (edema) swelling.

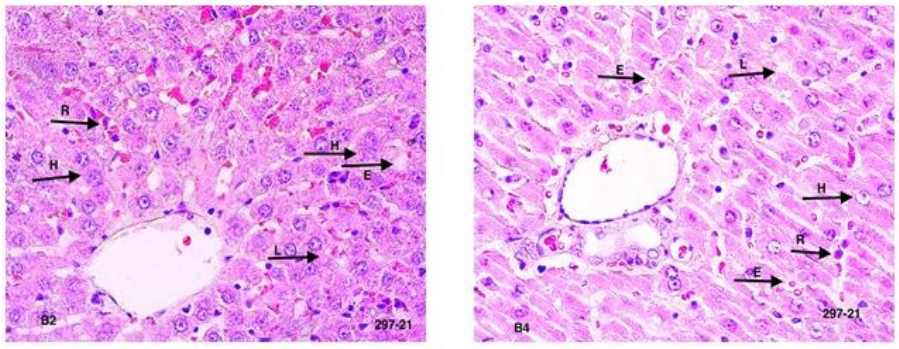

Figure 3. Histopathology of the liver of male white rats magnified 400X with H&E staining score 2 (moderate damage)

Arrows marks, indicated : moderate damage. Necrotic cells 25-250 %. H (hepatosid) necrotic. R (inflammation) Inflammatory cells. E (edema) swelling moderate damage. Necrotic cells 25-250 %. H (hepatosid) necrotic. R (inflammatory cells). E (edema) swelling. L (fat) fat degen .

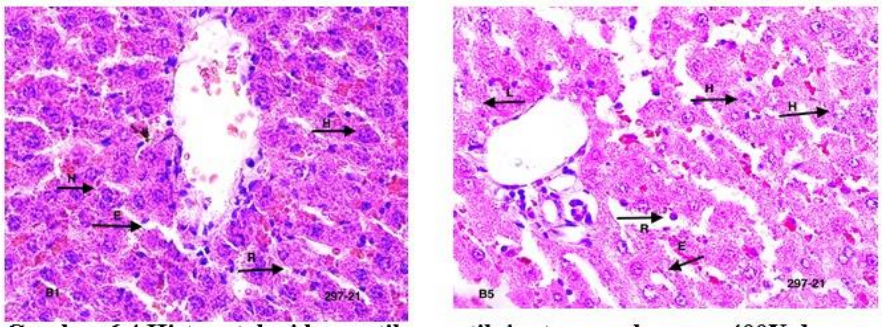

Figure 4. Histopathology of the liver of male white rats magnified 400X with H&E staining score 3 (severe damage)

Arrows marks, indicated : heavy damage. Necrotic > 50%. H (hepatosid) necrotic. R (inflammation) Inflammatory cells. E (edema) swelling. L (fat) fat degeneration severe damage. Necrotic > 50%. H (hepatosid) necrotic. R (inflammation) Inflammatory cells. E (edema) swelling. L (fat) fat degen

Table 1. shows that the negative control and treatment group at a dose of 125 mg/kg BW experienced the highest level of damage among all treatments. This shows that there are necrotic cells > 25%-50%, necrotic hepatocytes, inflammation of inflammatory cells, swelling edema and fatty degeneration of liver cells. The visible inflammatory cells are thought to be due to liver cell injury that causes acute or chronic inflammatory cell influx to the liver. Attacks on live liver cells that express antigens by sensitized T cells are a common cause of liver damage (Kumar, 2007). In addition, the occurrence of fatty liver (steatosis) is the accumulation of fat in the liver cells. Stetatosis is generally caused by toxins, protein malnutrition, diabetes mellitus (DM), obesity and anoxia. The most common causes of fatty liver are alcoholic and non-alcoholic. The most common non-

alcoholic causes are diabetes and obesity. In this study, fatty liver was thought to be caused by a toxin caused by streptozotocin administration.

Streptozotocin has a cytotoxic effect that can damage pancreatic beta cells (Goodman & Gilman, 2008). In pancreatic beta cells, streptozotocin damages DNA through the formation of NO, hydroxyl radicals, and hydrogen peroxide, all of which are free radical compounds that very quickly damage cell tissue. Liver cells are the main tissue that is the target of increased concentrations of free radicals because the liver is the site of metabolism of xenobiotic compounds (Salim, Nur., & Balqis, Umm, 2017).

Table 2. The Result *Mann Whitney histopathological scoring*

Treatment Group	Normal	Negative	Positive	Dose of 125 mg/kg BW	Dose of 150 mg/kg BW	Dose of 175 mg/kg BW
Normal		0.005	0.005	0.005	0.005	0.005
Negatif	0.005		0.058	1,000	0.031	0.339
Positif	0.005	0.058		0.058	0.549	0,339
Dose of 125 mg/kg bw	0.005	1.000	0.058		0,031	0.339
Dose of 150 mg/kg bw	0.005	0.031	0.549	0,031		0.166
Dose of 175 mg/kg bw	0.005	0.339	0.339	0.339	0.166	

The results of the Mann-Whitney test analysis showed that there was a significant difference in liver histopathological scoring from each treatment group, namely the treatment group giving pumpkin leaf extract 125 mg/kg bw (dose 1), 150 mg/kg bw (dose 2) and 175 mg/kg bw (dose 3) was significantly different ($p < 0.05$) with the normal control group, indicating that the level of improvement had not yet reached optimal. This is because the dose variation in pumpkin leaves has very little activity in regenerating damage to the liver of male white rats.

In the treatment group, dose 1 and dose 3 were significantly different from the negative control which stated that the dose 1 and dose 3 were as damaged as the negative control. Dose 1 has a score = 2.4, this score is higher than the score at dose 2 and dose 3. This is due to the calculation of the score based on the area of degeneration and darker colored cells. While at dose 2, it was not significantly different from the negative control. At dose 2 it has a score of = 1.4, this score is the lowest between dose 1 and dose 3. This score of liver cell damage has not shown any necrosis so that liver damage is

included in the mild category and causes enzymes not to leak cells and remain high in the liver. So that dose 2 has a fairly good activity in regenerating damaged liver cells in male white rats.

Liver cell damage caused by free radicals can be overcome by antioxidants. Antioxidants are compounds that have a free radical molecular structure without disturbing their function and can break the chain reaction of free radicals. In general, antioxidants inhibit fat oxidation. Flavonoids are an example of a natural antioxidant.

In this study, the dose that can regenerate liver cells is a dose of 150 mg/kg bw (dose 2). This is due to the content of secondary metabolites contained in pumpkin leaf plants such as flavonoids, saponins, and steroids that can regenerate damage to the liver of male white rats. Especially flavonoids as natural antioxidants work as active substances to protect the mucosa by preventing the formation of lesions by various necrotic agents. In addition, pumpkin is a cultivated plant that grows well in hot humid climates and likes open places and gets sunlight so that pumpkin will grow optimally. So that the nutrients contained in pumpkin plants are well enough. Phytochemical content in a plant is influenced by several factors such as light, temperature, humidity, pH, nutrient content in the soil and where it grows (Sholekah, 2017).

CONCLUSION

Based on the results of the study it can be concluded that:

1. Ethanol extract of pumpkin leaves *(C. moschata* Durch) contains secondary metabolites, namely alkaloids, flavonoids, saponins and tannins.
2. Ethanol extract of pumpkin leaves *(C. moschata* Durch)) at a dose of 150 mg/kg BW had an effect on the regeneration of liver cells of male white rats induced by streptozotocin.
3. The ethanol extract of pumpkin leaves *(C. moschata* Durch) did not have an effective dose that could regenerate the liver cells of male white rats.

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