

## Activity Test of *Cassia alata* L. Leaf Extract on Kidney Histopathology Damage Score of Diabetes Mellitus Model

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### ABSTRACT

Diabetic nephropathy is a disease experienced by diabetic patients. In this disease, damage to the glomerulus occurs. The administration of antidiabetic drugs tends to cause unwanted side effects, so there is a need for alternative treatment from herbs, one of which is *Cassia alata* L. *Cassia alata* L. leaves have chemical content such as flavonoids, alkaloids, tannins, and saponins. Flavonoids reduce blood glucose levels by increasing insulin secretion and mimetic agents, tannins slow carbohydrate digestion and saponins repair pancreatic beta cells and increase glycogen in the liver. This study aims to determine the effect of *Cassia alata* L. ethanol on the histopathology damage score of male white rat kidney (*Rattus norvegicus*) induced by streptozotocin. The test animals used were 30 male white rats divided into six treatment groups, each group consisting of five male white rats, namely the normal control group, negative control group, positive control group, treatment group with doses of 500 mg/kg BW, 600 mg/kg BW, and 700 mg/kg BW. The parameters evaluated were kidney tissue damage score and histopathology image analysis. The results showed that the ethanol extract of *Cassia alata* L. leaves contain alkaloids, flavonoids, saponins, and tannins. The ethanol extract of *Cassia alata* L. leaves in doses of 500 mg/kg BW, 600 mg/kg BW and 700 mg/kg BW has the activity of reducing the score of kidney tissue damage in male white rats and a dose of 700 mg/kg BW is the best dose in reducing the score of kidney tissue damage in diabetes mellitus models with a score of 2.

**Keywords:** *Cassia alata* L.; Damage Score; Histopathology; Kidney; streptozotocin

### INTRODUCTION

Diabetic nephropathy is a disease experienced by diabetic patients. In this disease, there is damage to the glomerulus. The damage to the glomerulus causes an unusual amount of blood proteins to be excreted in the urine. Under normal conditions, subatomic proteins cannot pass through the glomerulus, but under obsessive conditions, they can. The proximal tubules have the fundamental ability to reabsorb sodium, egg white, glucose, and water, and assist in the reutilization of bicarbonate. The proximal round epithelium is the part that is often affected by ischemia or exposed to toxins because the damage occurs due to high metabolic rates (Wirawan, 2018).

The most common factor causing diabetic kidney disease is consuming foods that are high in fat (lipids) and protein, this type of food will cause the performance of the kidney organs to increase from their normal limits to filter out food substances that are not needed by the body. From eating - uncontrolled food enters the body which causes diabetes mellitus so that glucose in the blood increases (hyperglycemia) because it cannot

be processed or utilized into energy (Kurniawati & Asikin, 2018). Hyperglycemia in diabetes mellitus can cause glucose autooxidation, protein glycation, and activation of polyol metabolic pathways which further accelerate the formation of reactive oxygen species (ROS) or the formation of oxidative stress. The presence of ROS causes the formation of free radicals in the body to increase. These free radicals can damage various body tissues, one of which is kidney tissue (Nurhikmah, 2018).

Treatment of diabetes mellitus is carried out by administering antidiabetic drugs such as glibenclamide and metformin or changing the patient's lifestyle. However, the administration of antidiabetic drugs may causes many unwanted side effects. Therefore, treatment of diabetes mellitus can be altered using herbs, such as *Cassia alata* L. *Cassia alata* L. leaves have chemical content such as flavonoids, alkaloids, tannins, and saponins. Flavanoids reduce blood glucose levels by increasing insulin secretion and mimetic agents, tannins slow carbohydrate digestion saponins repair pancreatic beta cells, and increase glycogen in the liver (Listani, 2018). Previous research on *Cassia alata* L. by Sugumar et al., (2016) concluded that *Cassia alata* L. leaves can prevent liver and kidney tissue from damage caused by oxidative

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stress during diabetes mellitus. Previous research conducted by Herlina (2019) concluded that from the results of statistical tests *Cassia alata* L. leaves were able to reduce blood glucose levels by 70% at a dose of 800 mg/kg BW.

Based on this, it is necessary to conduct research on *Cassia alata* L. on the score of kidney histopathology damage using diabetes model male white rat test animals with a dose variation, namely *Cassia alata* L. leaf extract 500 mg/kg BW, 600 mg/kg BW and a dose of 700 mg/kg BW.

## MATERIALS AND METHODS

### Materials

*Cassia alata* L. leaves were determined at UPT. Biological Resources Tadulako University, Glibenclamide, aqua pro injection, streptozotocin 40 mg/kg BW, glucometer, rotary evaporator, microscope, a set of minor surgical tools; scalpel (*different knife*) operating scissors (*scissors for surgery*), anatomy tweezers (*to clamp tissue or organs*), Male White Rats aged 2 months with a body weight (BW) range of 150-180 gram. The research was approved by the Research Ethics Committee of Universitas Tadulako under protocol number 1179/UN28.1.30/KL/2022/ to ensure compliance with ethical standards.

### Methods

#### Preparation of *Cassia alata* L. leaves ethanol extract

*Cassia alata* L. leaves extract is made by maceration technique. *Cassia alata* L. leaves powder that has been filtered using a cross-section filter mesh 40, weighed 1000 grams then put into 2 maceration containers 500 grams each containing 96% ethanol which can dissolve as much as 3 liters per 1.5 liters, closed, then left for 3 x 24 hours protected from light while stirring occasionally. The extract is then taken using channel paper to get the filtrate. Then at that time, it was concentrated using a Turning Vaccum Evaporator at a temperature of 40-60°C followed by drying which ended with a water bath at 60°C to obtain a thick concentrate.

#### Preparation of Glibenclamide Suspension and Streptozotocin Solution

The adult dose of Glibenclamide is 5 mg per day, so the dose of metformin for male white rats is 0.45 mg/200 kg bw. Weigh the Glibenclamide tablet powder, then suspend it in 0.5% NaCMC to 100 ml, then shake until homogeneous. Streptozotocin was weighed as much as 0.32 grams then dissolved using citrate buffer solution with a pH of 4.5, then induced intraperitoneally in rats. The dose of streptozotocin is 40 mg/kg bw.

#### Preparation and Testing of Histopathology of kidney

The surgical process was carried out on the abdominothoracal section, and a necropsy of the pancreatic organs was carried out, the organ was then rinsed with 0.9% NaCl physiological fluid to separate it from the blood or fats attached to the organ (Tandi, 2020). 3. Fixation Tissue samples were fixed with Buffered Neutral Formalin (BNF), the volume of Buffered Neutral Formalin (BNF) was at least 10 times the tissue volume. In general, the time needed for perfect fixation is 48 hours. The specimen selected for examination is cut 0.5-1 cm thick. Pieces of specimens are put in the processing basket accompanied by a label with a specimen number written in pencil. The remaining specimens with Buffered Neutral Formalin (BNF) were stored in tightly closed bottles. Furthermore, these bottles are stored sequentially and discarded when they have exceeded 3 months as written on the sample destruction form. The processing is conducted starting from the fixation process to preserve and harden the organs, the dehydration process to remove all the fluid contained in the fixed tissue, the clearing process to remove alcohol from the organs, and the impregnation process to remove toluene from the organs.

The embedding cassette which has been filled with organ specimens is inserted into the tissue processor with a time setting. The embedding cassette is removed from the tissue processor and continues with the embedding process. The embedding process is carried out to harden the organs so they can be easily cut using a microtome. Print is numbered along with a label so as not to be confused. After freezing (the paraffin has hardened) separate the mold from the basket. The next process is cutting with a microtome knife. The cutting process is carried out to obtain thin slices using a microtome. The process of cutting a network block is as follows. Take the network block then fix it on the microtome. The tissue block was cut with a coarse microtome to obtain a flat surface. Use a microtome knife that is still sharp, the thickness of the piece is 5-6 microns.

Select the best piece of tissue from the bands formed. The selected pieces are stretched in a floating out which has a temperature of around 40°C which is more. The ideal temperature will cause the pieces of tissue to stretch perfectly, not wrinkled. Sprinkle 5 grams of gelatin powder with 100 cc of distilled water and let it dissolve completely. Good cuts, not scratched, nor wrinkled are selected and taken with glass slides that are numbered according to the pathology number. The slide containing the patch of tissue is placed on the slide heating plate, for a minimum of two hours.

The staining process is conducted to give color to the tissue that has been cut so that the tissue becomes contrasted so that it can be recognized and observed with a microscope. Before coloring, all dyes must be checked for clarity. After the coloring is done cover slipping, prepare enough cover slips by the amount of the preparation that has just been colored then put 1-2 drops of "entellan" on each cover slip. Turn it over and cover it on the slide that has just been colored, preventing air bubbles from forming, let the preparation that has been covered with a coverslip then leave it until it dries completely. Clean the slide glass with xylol and then give the number according to the number on the slide glass label and it is ready to be examined under a light microscope (Luna, 1968). Readings are taken under a microscope to see changes in the morphology of the organ being examined. The examination was carried out using an Olympus CX-23 microscope (Luna, 1968).

#### Data Analysis

Primary data collected in this study included the results of examining the histopathological picture of the kidneys of male white rats (*Rattus norvegicus*) in the form of histopathological scoring, namely score 0: normal; score 1: kidney tubule damage 1-25%; score 2: kidney tubule damage 25-50%; score 3: kidney tubule damage more than 50-75%; and score 4: kidney tubule damage more than 75-100%. Data obtained in the form of kidney damage scoring were analyzed using the Kruskal-Wallis non-parametric test at the 95% confidence level if there were significant differences between groups, followed by the Mann-Whitney test to further understand the location of significant differences in the test groups.

#### RESULTS

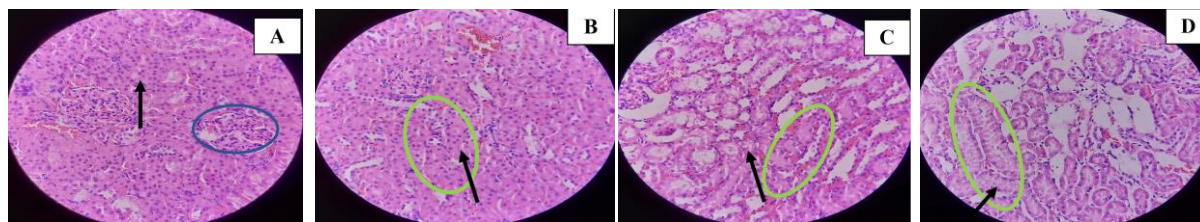
The test material in this study was *Cassia alata* L. leaves obtained from the city of Palu. The determination aimed to determine the accuracy of the test material employed. Determination of *Cassia alata* L. leaves were carried out at UPT. Biological Resources Tadulako University, Central Sulawesi. The analysis revealed that the *Cassia alata* L. leaves utilized in the study belonged to the family Basellaceae. The bioactive secondary metabolites present in the ethanolic extract which expected to have a role in delivering nephroprotective effects were identified through phytochemical screening. The ethanolic extract of *Cassia alata* L. leaves contained positive alkaloids, flavonoids, saponins, tannins, and phenolics, according to the results of phytochemical

screening (Table I). *Cassia alata* L. leaves extract contained a wide range of polar, semi-polar, and non-polar secondary metabolite chemicals.

Based on the scoring data on the level of kidney damage, the average level of damage in the normal control has the lowest score with an average damage value of 0. This is because the normal control does not get the treatment of streptozotocin administration. In normal control (Figure 1.A), there are no changes in either tubulus or glomerulus and the cells look all normal. The negative control has a remarkably high score with an average damage value of 3. This is because the negative control gets streptozotocin induction and does not get the therapeutic effect of chemical drugs and *Cassia alata* L. It is also seen 50 - 75% damage to cells, where tubule cells atrophy with coating cells experiencing necrosis as much as more than 2/3 of the field of view (green circle). Cell necrosis is characterized by pale cytoplasm and the nucleus shrinks or disappears (black arrow). The positive control has a score of 1.4. This is because the positive control was treated with streptozotocin induction and *Cassia alata* L. so that there was <25% damage to the cells, where the tubule cells atrophied with the coating cells experiencing necrosis as much as 1/3 of the field of view (green circle). Cells experiencing necrosis are characterized by rather pale cytoplasm, and basophilic nuclei but rather pale (black arrow). At the dose of 500 mg/kg BW, 600 mg/kg BW has a damage score of 2.4 and the dose of 700 mg/kg BW has a damage score of 2. This is because the variation of the three doses gets *Cassia alata* L. leaf therapy so that cell repair occurs, where 25 - 50% damage is seen in cells.

#### DISCUSSION

From the results of histopathological observations and statistical tests, it can be seen that the most effective dose of ketepeng leaves in regenerating kidney cells is a dose of 700 mg/kg BW. This is because the 700 mg/kg BW dose uses more *Cassia alata* L. leaf than other dose variations so that the secondary metabolite compounds contained therein are better at regenerating kidney cells. There is an improvement in kidney cells due to secondary metabolites contained in *Cassia alata* L. leaf here secondary metabolites compounds are efficacious as antioxidants that neutralize free radicals that cause diabetes mellitus including flavonoids, saponins, and alkaloids which are secondary metabolite compounds with strong antioxidant activity. According to research conducted by (Sugumar et al., 2016) states that *Cassia alata* L. leaves tend



**Figure 1. Histopathology of White Rat Damage Score with HE Stains, 400X Magnification.**

Description: A = Scoring 0: there is a glomerulus within normal limits (blue circle) appears normal tubules consisting of cells with eosinophilic cytoplasm and basophilic nuclei (black arrow); B = Scoring 1: atrophic tubules with coating cells experiencing necrosis as much as 1/3 of the visual field (green circle). Cells experiencing necrosis are characterized by slightly pale cytoplasm, and basophilic nuclei but slightly pale (black arrow); C = Scoring 2: atrophic tubules with the lining cells experiencing necrosis as much as 2/3 of the visual field (green circle). Cells experiencing necrosis are characterized by slightly pale cytoplasm and shrinking nuclei (black arrows); D = Scoring 3: atrophic tubules with more than 2/3 of the lining cells necrotizing (green circle). Cell necrosis is characterized by pale cytoplasm and shrunken or missing nuclei (black arrow).

**Table I. The Results of Phytochemical Screening of *Cassia alata* L. leaves Extract**

Chemical Compounds	Reagent	Result	Exp.
Alkaloids	Dragendorf	Red	+
Flavonoids	Mg powder + HCl	Red yellow	+
Saponins	Water + HCl 2N	Foam ± 1 cm	+
Tannins	FeCl <sub>3</sub> + NaCl 10%	Blackish green	+
Steroid	Chloroform, anhydrous acetic acid, sulfuric acid	Formed Bluish Green	-

Note: (+) : Detected; (-) : Not Detected

**Table II. The Results of Histopathology Damage Score Examination**

Groups	Damage Score Examination					Average ± SD
	1	2	3	4	5	
Normal	0	0	0	0	0	0 ± 0.000
Negative	3	3	3	3	3	3 ± 0.000
Positive	1	1	1	2	2	1.4 ± 0.548
500 mg/kg BW	2	3	3	3	1	2.4 ± 0,894
600 mg/kg BW	3	3	1	3	2	2.4 ± 0.894
700 mg/kg BW	1	2	3	2	2	2 ± 0.707

Description: 0 (Normal); 1 (Tubullus injury < 25%); 2 (Tubullus injury 25 - 50%); 3 (Tubullus injury 50 - 75%); 4 (Tubullus injury >75%)

to prevent liver and kidney tissue from damage caused by oxidative stress damage caused by oxidative stress during diabetes mellitus. It has a good impact on enzymatic antioxidants on both enzymatic (SOD, CAT, GPx, and GST) and nonenzymatic (vitamin C, vitamin E, and GSH). It strongly affects the rate of protein degradation by reducing the elimination of its end products (urea, uric acid, and creatinine).

### CONCLUSION

The results of the study showed that the ethanol extract of *Cassia alata* L. leaves at a dose of

700 mg/kg BW with an average damage of 2 was an effective dose in repairing kidney cells, in male white rats and able to repair kidney tissue damage in male white rats.

### CONFLICT OF INTEREST

No conflict of interest.

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