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Conference Paper

PROTECTION OF DAYAK ONION TUBER EXTRACT (*Eleutherine palmifolia*) AGAINST KIDNEY HISTOPATHOLOGICAL APPEARENCE OF ALBINO MALE RAT STRAIN WISTAR WHICH WAS INDUCED BY ALLOXAN

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Abstract

The purpose of this study was to know the effect of Dayak onion tuber extract (*Eleutherine palmifolia*) given by per oral in lowering levels of histophatology damage kidney of albino male rat (*Rattus norvegicus*) strain Wistar which was induced by alloxan. Animals which were used in this research were 24 white male rats (*Rattus norvegicus*) strain Wistar, and then divided into 6 groups. The negative control group K (-) were given with aquabidest and CMC-Na 1% during the therapy period, the positive control group K (+) were given with alloxan 110 mg / kgbw, the group of drug control K (0) were given with alloxan and oral therapy with metformin 9 mg / 200g bw / day, the treatment group 1 (P1) were given with alloxan and per oral therapy with extract of Dayak onion tuber 100 mg / kgbw, the treatment group 2 (P2) were given with alloxan and per oral therapy with extract of Dayak onion tuber 200 mg / kgbw and the treatment group 3 (P3) were given with alloxan and per oral therapy with extract of Dayak onion tuber 400 mg / kgbw. The therapy was given for 14 days, then the animals were sacrificed with ketamine and then its kidney was taken for examination of hisphatology in kidney. Observations based on their depiction of renal histopathology tubular degeneration and necrosis, glomerular necrosis, intestitial infiltration and glomerular sclerosis. Data obtained from the scoring of histopathological appearence albino rat kidneys were analyzed by test *Kruskal-Wallis* and if there is a real difference followed by *Mann-Whitney* test using SPSS 20.0 for windows. The results showed that the extract of Dayak onion tuber 400 mg/ kgbw can reduce the degree of kidney damage in albino male rat exposed to alloxan significantly.

Keywords: *Eleutherine palmifolia,* alloxan, kidney, histhopathology.

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1. Introduction

Diabetes mellitus has low concentrations of the hormone insulin, so blood sugar levels will be high. In the condition of high glucose levels in the blood can cause the formation of Reactive Oxygen Species (ROS) that result in oxidative stress. The role of insulin is to regulate blood sugar levels. Hyperglycemia or elevated blood sugar levels, is an uncontrolled effect of diabetes and in the long term can cause serious damage to some body systems, especially in the heart blood vessels (coronary heart disease), eyes (can occur blindness), nerves (may occur stroke), kidney (can occur kidney failure). [1, 2].

Diabetic nephropathy is a chronic microvascular complication of diabetes mellitus in the kidney that has a variety of adverse effects and is a leading cause of end-stage renal disease characterized by urinary albumin excretion through urine and a decrease in glomerular filtrartion rate (GFR) filtration rate [3]. Histopathologically in diabetic nephropathy includes changes in the glomerulus of the glomerular capillaries of basal membrane and capsule, changes in the renal vascular membranes of arteriosclerosis, changes in the tubules and interstitials that may be hyaline deposits of the proximal tubules, glycogen deposits of the proximal tubules and tubular atrophy. [4]. Treatment of diabetes mellitus can be treated with oral medication Usage of oral medication commonly used is oral hypoglycemic drugs biguanida like metformin. Chemical drugs usually have side effects. As a result of side effects caused by chemical drugs consumed, it is necessary the existence of alternative antidiabetic drugs without side effects, cheap and easily accessible by the community. [5, 6].

One of the alternative treatments to replace chemical drugs is to use traditional medicinal plants that can prevent and treat diabetes mellitus. [6] Onion Dayak or ghost onion (Eleutherine palmifolia (L.) Merr) is a typical plant of Central Kalimantan. This plant has been used for generations of Dayak community as a medicinal plant. In Dayak bulbs there are phytochemical compounds namely alkaloids, glycosides, flavonoids, phenolics, steroids and tannins [7]. Judging from its chemical content, potency of Dayak bulb as a medicinal plant multifunction is very big. Of these, the high antioxidant is flavonoids. The compound of this polyphenol derivative is capable of stabilizing and removing unpaired electrons and capable of counteracting free radicals [8].

2. Materials and Methods

2.1. Induction of Aloxan

Alloxan induction was performed on the first day after 3 days of adaptation. Alloxan dose of 110mg / kgBW is intraperitoneally and dissolved in aquabidestila.

2.2. Giving Metformin

Metformin administration was performed for 14 days after alloxan induction after adaptation for seven days. Metformin dose 9mg / 200g orally and dissolved into CMC-Na.

2.3. Giving Dayak Banana Extract Therapy

Dayak extract of Dayak (Eleutherine palmifolia) extract was performed for 14 days after alloxan induction after adaptation for seven days. The dosage of Dayak bulb extract (Eleutherine palmifolia) 100, 200 and 400mg / kgBB peroral and each dose of extract will be dissolved in CMC-Na in 3 treatment groups.

2.4. Sampling for Histopathology Preparations

Intake of the kidneys was performed on the 18th day after treatment, by euthanation with ketamine 3mg / kgBB, then performed abdominal surgery for further kidney organ retrieval and inserted into a closed plastic pot with 10% formalin content.

2.5. Collection and Data Collection Techniques

Histopathological data were determined by scoring methods according to the method a modified Klopfleisch score in Table 1 [9].

2.6. Research Design and Data Analysis

The type of research used is laboratory experimental. The research design used was Completely Randomized Design (RAL). Data are presented in the mean ± standard deviation (mean ± SD). Data are arranged in tabular form and then analyzed statistically by using Kruskal-Wallis test. If there was a marked difference between the study groups ($p < 0.05$), followed by the Mann Whitney test. [10]

Table 1: Histopathology lesions observed in kidney rat.

SCORE	Degeneration of tubular epithelial cells
0	No degenerative changes occur
1	If the number of degenerative cells <25% of the field of view
2	If the number of degenerative cells between 26 - 50% of the field of view
3	If the number of degenerative cells between 51 - 75% of the field of view
4	If the number of degenerative cells> 76% of the field of view
SCORE	**Tubular epithelial necrosis**
0	No necrotic changes
2	If the number of necrotic cells <25% of the field of view
4	If the number of necrotic cells is between 26 - 50% of the field of view
6	If the number of necrotic cells is between 51 - 75% of the field of view
8	If the number of necrotic cells is> 76% of the field of view
SCORE	**Glomerular necrosis**
0	If there is no change of necrosis in glomerular cells
3	If glomerular necrosis <25% of all glomeruli
5	If glomerular necrosis is 26 - 50% of all glomeruli
7	If glomerular necrosis 51 - 75% of all glomeruli
8	If glomerular necrosis is> 76% of all glomeruli
SCORE	**Interstitial infiltration**
0	If no inflamed cell is found in the interstitial space
1	If inflammation cells are found <25% of the interstitial space
2	If inflammation cells found 26 - 50% of the interstitial space
3	If inflammation cell finds 51 - 75% of the interstitial space
4	If inflammation cells are found> 76% of the interstitial space
SCORE	**Glomerular sclerosis**
0	If there is no proliferation of funds or glomerular tau sclerosis
1	If glomerular pluriferation / sclerosis <25% of all glomeruli
2	If glomerular pluriferation / sclerosis is 26 - 50% of all glomeruli
3	If glomerular pluriferation / sclerosis 51 - 75% of all glomeruli
4	If gloriular ploriferation / sclerosis is> 76% of all glomeruli

3. Results

4. Discussion

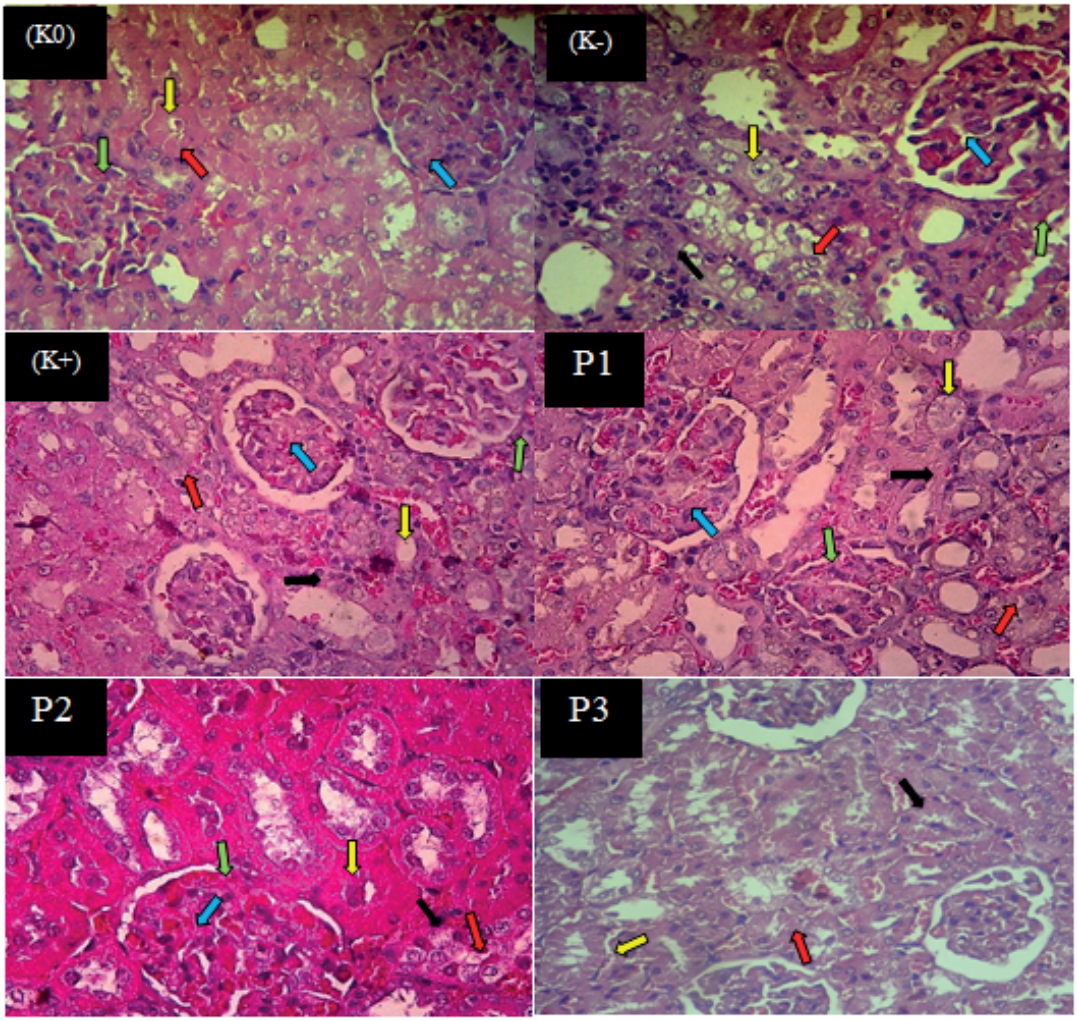

Figure 1: Histopathology of 400x magnification renal dye with HE staining, examined with Olympus CX-41 microscope, tubular degeneration (red arrow), tubular necrosis (yellow arrow), glomerular necrosis (green arrow), interstitial infiltration (black arrow), glomerular sclerosis (blue arrow).

Table 2: Mean and Standard Deviation On Each Variable and Treatment.

Treatment	Mean ± SD				
	Degeneration of the renal tubules	Kidney tubular necrosis	Glomerular necrosis	Interstitial infiltration	Glomerular sclerosis
(Ko)	$1{,}10^{a}$ ±0,115	$0{,}75^{a}$ ±0,251	$1{,}30^{a}$ ±1,089	$0{,}00^{a}$ ±0,000	$0{,}40^{a}$ ±0,282
(K-)	$3{,}90^{d}$ ±0,200	$5{,}70^{d}$ ±0,200	$5{,}80^{c}$ ±1,608	$1{,}15^{c}$ ±0,718	$3{,}32^{c}$ ±0,680
(K+)	$3{,}25^{c}$ ±0,341	$3{,}10^{c}$ ±0,945	$5{,}05^{c}$ ±0,300	$0{,}60^{bc}$ ±0,489	$2{,}20^{b}$ ±0,952
P1	$2{,}95^{bc}$ ±0,660	$2{,}70^{c}$ ±0,886	$4{,}80^{bc}$ ±1,751	$0{,}15^{ab}$ ±0,300	$2{,}70^{bc}$ ±0,200
P2	$1{,}95^{ab}$ ±0,680	$2{,}10^{bc}$ ±0,326	$2{,}65^{ab}$ ±1,279	$0{,}35^{b}$ ±0,251	$1{,}70^{b}$ ±1,051
P3	$1{,}35^{a}$ ±0,341	$1{,}20^{ab}$ ±0,326	$1{,}05^{a}$ ±1,112	$0{,}30^{b}$ ±0,258	$0{,}30^{a}$ ±0,382

*Different superscripts in the same location showed a marked difference ($p < 0.05$).

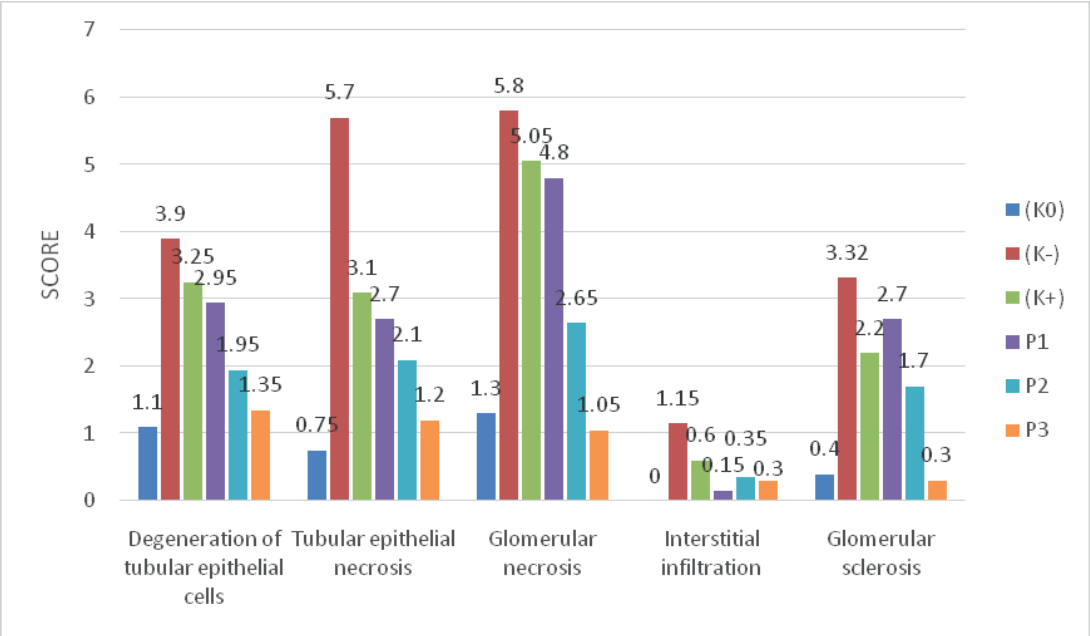

Figure 2: The Mean Graph of Histopathology overview of renal male rat in each treatment.

4.1. Degeneration of the Kidney Tubule

The negative control group (K-) has the highest rates of renal tubular degeneration compared with the other treatment groups caused by alloxan in the body undergoing reduction oxidation metabolism resulting in free radical. [11] Circumstances cause free radical instability, are highly reactive and can damage living cells (cytotoxic). This process causes cell function is not optimal and in the long term can occur degeneration. [12]

The positive control group (K +) given oral medication of metformin therapy had a high rate of renal tubular degeneration from the Dayak Dayak extract treatment group, because by administering antidiabetic drugs when a decline in renal function could result in drugs excreted through the kidneys it would accumulate and could have an effect toxic or may aggravate the condition of the kidney. [13] The P1 treatment group treated with Dayak 100bg / kgBB Dayak bulb extract had higher mean of renal tubular degeneration compared with treatment group P2 and P3. This is because the high doses in the P2 and P3 groups contain many active compounds in the form of polyphenols and flavonoids contained in Dayak bulbs have the activity of providing hydrogen ions capable of cleaning free radicals. [8]

4.2. Kidney Tubular Necrosis

The negative control group (K-) had the highest mean renal tubular necrosis compared with the other treatment groups. Diabetes mellitus causes the release of calcium ions from the mitochondria resulting in homeostatic disorders that are the beginning of the death of the cell (necrosis). [14] The P3 treatment group that was given Dayak 400gg / kgBB Dayak bulb extract therapy had mean number of necrosis of renal tubular cells higher than those of P1 and P2. Triterpenoid compounds are known to have high antioxidant activity capable of counteracting free radicals by releasing hydrogen atoms from their hydroxyl groups so as to convert them to stabilize. [15]

4.3. Glomerular Necrosis

The negative control group (K-) had the highest mean glomerular necrosis rate compared with the other treatment groups. In hyperglycemic states there is an increase in the production of lipid peroxidation of lipid peroxide and glomerular mesangial cell hydrogen peroxide. [16] The P3 treatment group treated with Dayak Dayak bulb extract 400mg / kgBB had a lower mean glomerular necrosis number than P1 and P2. The content of flavonoids and tannins acts as an antioxidant that can reduce the disorder of renal pathology due to diabetes mellitus, so the more active compound content can reduce the level of pathology in the form of necrosis of the glomerulus. [17]

4.4. Interstitial Infiltration

The negative control group (K-) had the highest mean infiltration rate of inflammatory cells compared with the other treatment groups. This suggests that the renal cell constituent cells can be damaged when alloxan is induced, since alloxan is an unstable and selectively toxic hydrophilic compound to the liver and kidneys. [18]

In the treatment group P1, P2 and P3 infiltrate of inflammatory cells in the interstitial tubules were not significantly different but there was an increase in the number of inflammatory cells, indicating that inflammation (an inflammatory reaction) is an important mechanism for defending from harm, such as pathogenic agents, dead or damaged cells, or irritation, so that inflammation is a protective effort by the body to capture harmful stimuli and to initiate the healing process. [19]

4.5. Glomerular Sclerosis

The negative control group (K-) had the highest mean sclerotic glomerular number compared with the other treatment groups. gave significantly different results than other treatments. Diabetic nephropathy causes some abnormalities in the histologic structure of the kidney. Histologic characteristics of diabetic nephropathy are structural changes in the glomerulus. Glomerulus in the state of diabetic nephropathy increases volume (hypertrophy) caused by extracellular matrix buildup, thickening of the glomerular basement membrane, and glomerulosclerosis. [20] In the (K +) group, P1 and P2 showed that the sclerosis present in the glomerulus was not significantly different. This is probably due to the less metformin improving the structure of the kidneys due to the administration of antidiabetic drugs at the time of decline in renal conditions can be toxic or worsen the state of the kidney and due to the lack of active flavonoid compounds contained in Dayak bulbs on P1 (100mg / kgBB) and P2 (200mg / kgBB).

5. Conclusion

Based on the research that has been done, it can be concluded that the giving of Dayak bulb extract (Eleutherine palmifolia L., Merr) with dose of 400mg / kgBB can affect the alloxan induced histopathology of rats (Rattus norvegicus).

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