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Effect of Red Betel Leaf Extract (Piper Crocatum) on Blood Glucose Levels and Kidney Histopathology in Streptozotosin-Nicotinamide Induced Streptozotosin-Nicotinamide Rats

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Abstract

Diabetic nephropathy is a complication of diabetes mellitus which results in changes in the structure and function of the kidneys. Red betel leaf (Piper crocatum) can be used as a treatment for diabetic nephropathy, which has antioxidant properties. This study aims to determine the optimal dose of ethanol extract of red betel leaves, the effect of ethanol extract of red betel leaves on blood glucose, improvement of kidney function and kidney histopathology in STZ-NA induced rats. This study used 30 male white rats of the Wistar strain, 5 groups were conditioned to experience hyperglycemia and waited for three weeks after induction of STZ-NA via intra peritoneal 60 mg/kg BW and 120 mg/kg BW. After it was stated that the rats had diabetic nephropathy, they were given an oral test preparation. Parameters measured were blood glucose, BUN, and renal histopathological tests. Data analysis used the ANOVA method followed by the LSD Post Hoc test. The test results of antihyperglycemic activity and improvement of kidney function stated that the ethanol extract of red betel leaves at doses of 125 mg/kg BW and 250 mg/kg BW had a significant difference between the normal group, negative control and positive control, but at a dose of 500 mg/kg BW there was no difference significantly with the positive control where the ethanol extract of red betel leaves has the same effect as the positive control. The results of the renal histopathological test at a dose of 250 mg/kg BW were significantly different from the normal control, negative control, positive control, but at a dose of 500 mg/kg BW there was no difference from the positive group.

Introduction

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia and disorders of carbohydrate, fat, and protein metabolism that are linked to an absolute or relative deficiency in the function and/or secretion of insulin. Symptoms experienced by DM patients include polydipsia, polyuria, polyphagia, weight loss, and numbness. Poorly controlled DM can lead to acute and chronic complications. Chronic complications include microvascular complications such as nephropathy, diabetic retinopathy, and neuropathy (Saputri, 2020).

The number of cases of Type 2 DM is expected to increase by 300 million people globally in 2025. The increase in the incidence of Type 2 DM will be accompanied by an increase in the likelihood of chronic DM complications. The prevalence of Type 2 DM among white people ranges from 3-6% of the adult population. Some ethnic groups, such as the Polynesian

population in the Pacific, the Indian Pima in the United States, Mexico, the Creole population in Suriname, the indigenous population of Australia, and Indian immigrants in Asia, have a Type 2 DM incidence of up to 35% due to lifestyle changes. Type 2 DM in Indonesia is around 1.4-1.6% of the entire adult population based on epidemiological data. The World Health Organization (WHO) estimates that in 2025, Indonesia will be ranked fifth in the world with 12.4 million Type 2 DM patients. Various prospective studies have shown an increase in microvascular complications such as diabetic nephropathy, resulting in more DM patients needing dialysis than in previous decades. These complications can occur more quickly or possibly be delayed depending on each individual's glycemic status. The number of hospitalized cases of elderly DM patients at Immanuel Bandung Hospital from October 2010 - September 2011 was 420 cases with 30 cases of Diabetic Kidney Disease (DKD), namely 7.14%. Diabetic nephropathy accounted for 26.50% of cases in RSCM in 2011 (Halimah, n.d.).

In developing an animal model of DM leading to nephropathy complications, streptozotocin (STZ) and nicotinamide (NA) are used as diabetogenic agents. Administration of STZ-NA once with a dose of 60 mg/kg BW and 120mg/kg BW in rats can cause hyperglycemia. Long-term hyperglycemia can lead to diabetic nephropathy complications. It takes three weeks for the test animals to experience a decrease in kidney function (Rossing et al., 2022).

Long-term hyperglycemia activates Protein Kinase C (PKC) via diacylglycerol. Protein Kinase C then stimulates the activity of angiotensin, disrupting the glomerular filtration rate (GFR). Insulin growth factor-1 (IGF-1), VEGF, and endothelin-1 (ET-1) trigger hypertrophy of the renal tubules. Protein Kinase C can stimulate tumor necrosis factor-α (TNF-α) and nuclear factor kappa B (NFκB) as well as increase fibrotic activity, namely connective tissue growth factor (CTGF) and transforming growth factor-ß (TGF-β). Both fibrosis factors increase the proliferation of connective tissue and cause glomerular hypertrophy and mesangial expansion. A decrease in antioxidant enzyme function is also exacerbated by PKC, which stimulates the release of inflammatory cytokines that can cause glomerulosclerosis.

Kidney failure causes damage to the glomeruli and tubules which results in excess protein and increased urea in the blood (Sartika et al., 2018). Hyperglycemia is a major factor causing nephropathy, along with hypertension, genetics, and smoking habits (Papadopoulou-Marketou et al., 2017).

Therapy for type II diabetes patients usually involves the use of antidiabetic drugs such as sulfonylureas, meglitinides, biguanides, thiazolidinediones, α-glucosidase inhibitors, amylinomimetic glucagon-like peptide 1 (GLP-1) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium-glucose co-transporter 2 (SGLT2) inhibitors. Side effects often occur from the long-term use of antidiabetic drugs, including hypoglycemia, liver, kidney, gastrointestinal, or respiratory system disorders, given that DM is a chronic disease during pregnancy (Dipiro, 2020).

Red betel (Piper crocatum) is a plant that grows in Indonesia and has benefits as an herbal plant. Red betel is a medicinal plant that belongs to the Piperaceae family. Red betel can be used to treat diabetes, gout, hepatitis, hypertension, and eye inflammation (Fajarwati et al., 2019). The secondary metabolites found in red betel are flavonoids, alkaloids, tannins, and saponins. Flavonoids include flavones, flavonols, flavanonols, flavanols, isoflavones, aurones, catechins, anthocyanidins, and chalcones (Sudiana & Purwanto, 2019). Flavonoids are phenolic compounds that have roles as antioxidants, antidiabetics, anticancer, antiseptics, and anti-inflammatory. Ethanol extract of red betel leaves can act as an antioxidant that helps to reduce oxidative stress. The antioxidant compounds in red betel leaves can suppress excess free radicals that stimulate the release of pro-inflammatory cytokines, leading to structural and functional damage to the kidneys, by providing hydrogen atoms directly and increasing endogenous antioxidant activity, such as glutathione peroxidase (Ramadhan et al., 2019).

Based on in vivo research conducted by (Sudiana & Purwanto, 2019), the 70% ethanol extract of red betel leaves can lower blood sugar levels in male white rats (Rattus novergicus) with doses of 50 and 100 mg/kg body weight, and mice with doses of 100 and 200 mg/kg body weight induced by aloxan. The 70% ethanol extract doses used significantly influenced the glucose levels of rats given oral treatment with a sonde once a day for three weeks. The comparison was made with positive control of glibenclamide at 0.02% (dose of 1 mL/kg body weight) and metformin at 10 mg/kg body weight. The active compounds that play a role in reducing blood sugar levels in the research are alkaloids, saponins, tannins, and flavonoids.

The toxicity study of red betel leaf infusion was carried out orally with repeated doses of up to 1890 mg/kg in mice for 28 days. The results showed that kidney function such as plasma urea levels remained normal after administration, indicating that red betel leaves did not show kidney toxicity (Nasution et al., 2021).

Based on the description above, the author conducted a study using red betel leaf extract to determine its anti-hyperglycemic activity and pancreatic cell regeneration in STZ-NA-induced rats. The anti-hyperglycemic activity was indicated by an increase in rat body weight, a decrease in blood glucose and BUN levels, and an improvement in histopathological profiles in rat kidney organs. These parameters distinguish the study from previous research.

Methods

The research conducted is an experimental study aimed at determining the anti-hyperglycemic activity and kidney function improvement of red betel leaf ethanol extract. The research subjects were male Wistar rats aged 6-8 weeks with a weight range of 150-200 grams, normal activity, and in good health. The rats were obtained from the University Intercenter Laboratory for Nutrition, Gadjah Mada University, Yogyakarta.

The population used in this study was red betel leaves. The sample used in this study was red betel leaves obtained from Sepang Simin Village, Gunung Mas Regency, Central Kalimantan, randomly selected, fresh, and not rotten.

The equipment used in this study was a maceration tool consisting of a brown bottle, stir bar, filter paper, glass funnel, volumetric flask, rotary evaporator, spectrophotometer, rat blood glucose measuring device, syringe, centrifuge, microscope, and Sterling-Bidwell surgical instruments (scalpel, tweezers, knives, scissors, needles, and wax table), rotary microtome, object glass and deck glass, and Olimphus CH20 light microscope.

The test materials used in this study were red betel leaves taken from Sepang Simin Village, Gunung Mas Regency, Central Kalimantan, and extracted through maceration method, 96% ethanol (Brataco), streptozotocin 60 mg/kg BW dissolved in citrate buffer (0.1 M, pH 4.5), nicotinamide 120 mg/kg BW dissolved in normal saline, glibenclamide, glucose reagent, BUN reagent, and formalin PA, Haematoxylin staining solution, Eosin staining solution, formaldehyde, ethanol, xylene, and alcohol from Merck.

The statistical analysis was performed using the normal distribution test (Shapiro-Wilk) to determine whether the data was normally distributed or not. If $p>0.05$, the distribution is normal, and if $p<0.05$, the distribution is not normal. Non-parametric test was conducted using Kruskal-Wallis for data analysis, if sig>0.05 then H0 is accepted, and if <0.05 then H0 is rejected. If the data is normally distributed with $p>0.05$, then a one-way parametric analysis (ANOVA) is performed. The test is followed by a post-hoc test to determine if there are any differences between each treatment group.

Results and Discussion

Production of Red Betel Leaf Ethanol Extract

Ethanol extraction of 1000 g of red betel leaves was carried out by maceration method using 95% ethanol solvent aimed at withdrawing the chemical components contained in the simplicia. The extract yield was 120.203 g with a yield of 3.878% as shown in table 1.

Table 1. Results of Making Red Betel Leaf Ethanol Extract

Powder Weight (g)	Extract Weight (g)	Extract Yield (%)
1000	120,203	12,020

Identification of the chemical content of the ethanol extract of red betel leaves using a test tube

Identification of the chemical compounds of the ethanol extract of red betel leaves was carried out by qualitative tests using color reactions at Laboratory 9 of Setia Budi University. The results of the identification of the ethanol extract of red betel leaves showed the presence of chemical compounds in the form of flavonoids, tannins, saponins and alkaloids. This can be known by comparing the qualitative tests conducted by the literature (Arnida et al., 2018). The chemical compounds contained in red betel leaves are flavonoids in the form of flavones, flavonols, flavonones, flavanonols, flavonols isoflavones, aurons, catechins, anthocyanidins and chalcones (Sudiana & Purwanto, 2019).

Table 2. Results of Identification of Chemical Content of Red Betel Leaf Ethanol Extract Using Tubes

Chemical Content	Results	References	Conclusion
Flavonoids	The orange color of the amyl alcohol layer	(Tampil et al., 2013)	+
Tannin	A blackish green color forms	(Khoriah et al., 2018)	+
Saponins	Foam height 2 cm	(Khoriah et al., 2018)	+
Alkaloids	Turbidity or brown precipitate is formed	(Khoriah et al., 2018)	+

Results of Examination of Rat Body Weight

The test animals used in this study were male white rats of the Wistar strain aged 2 months with a body weight of 150-200 grams obtained from the Nutrition Laboratory, Center for Food and Nutrition Studies, Gadjah Mada University. Mice were divided into 6 groups where each group consisted of 5 rats whose body weight was weighed.

The rats used were first acclimatized for 7 days then fasted and weighed to determine body weight. In the table below, the mice were weighed before being induced by STZ-NA. STZ induction at a dose of 60 mg/kg BW and NA 120 mg/kg BW was carried out intra peritoneally through the abdominal peritoneal cavity. STZ-NA was given to each treatment group except the normal group. Three days after administration of STZ-NA, weight was measured and left for 24 days so that it experienced a long-term hyperglycemia condition which caused complications of diabetic nephropathy (Rossing et al., 2022)

Table 3. Average Body Weight of Mice

Group	Average Rat Body (grams)		
	Day 24	Day 31	Day 39
I	206.8±5,11bc	212,8±4,54bc	219±4,63^{bc}
II	168,6±4,44a	164±4,84a	157,6±5,59^{ac}

III	166,2±3,96a	171,8±4,08a	176,8±4,08^{ab}
IV	165,6±4,39a	168,2±5,01a	171,6±4,82^{ab}
V	165,8±3,11a	170,6±3,43a	176±3,39^{ab}
VI	165,2±4,81a	170±4,84a	176,2±5,26^{ab}

Examination Results of Blood Glucose Levels

Blood glucose levels were measured before STZ-NA induced to determine initial blood glucose levels in mice. Blood glucose levels were then measured again on the 24th day when the rats had complications. The occurrence of DM in the induction group was characterized by hyperglycemia with glucose levels above 200 mg/dL. Blood glucose data can be seen in table 4.

Table 4. Average Blood Glucose Levels

Group	Day	Day	Day	AUC $^{24-39}$	
	The 24th	The 31th	The 39th		% Activity
I	72,48±1,25^{bc}	73,69± 0,98^{bc}	75,30± 1,81^{bc}	37,65	
II	258,65±1,67^{ac}	262,19± 1,73^{ac}	263,72± 2,52^{ac}	131,86	0,00
III	205,26±4,14^{ab}	123,33± 3,62^{ab}	91,82± 3,61^{ab}	45,91	59
IV	201,43±5,37^{ab}	137,16± 4,20^{abc}	131,82± 3,35^{abc}	65,91	48,8
V	200,90±7,94^{ab}	131,06± 6,31^{abc}	111,98± 6,42^{abc}	55,99	53,7
VI	204,43±1,97^{ab}	127,44± 1,77^{ab}	95,87± 1,68^{ab}	47,93	57,5

Blood Ureum Nitrogen (BUN) Examination Results

Measurement of Blood Ureum Nitrogen (BUN) was carried out before the rats were conditioned in a state of DM. BUN levels showed that the rats were still in a normal state. This was because the rats in each group had not received any treatment so that each rat had not experienced DM. After measuring BUN levels, then the test animals were induced by STZ-NA intraperitoneally to condition the rats in a state of DM and this would further cause damage to kidney function. Every rat in each group was induced by STZ-NA except for the normal group.

Table 5. Average Blood Ureum Nitrogen (BUN)

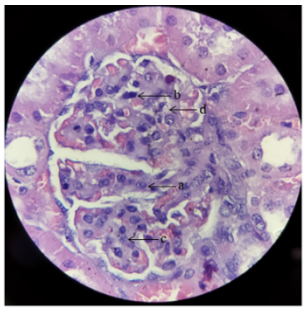
Group	Blood Urea Nitrogen (BUN) Levels (mg/dL)				
	Day 24	Day 31	Day 39	AUC $^{24-39}$	% Activity
I	10,88±0,16^{bc}	11,11.±0,20^{bc}	11,60±0,26^{b}	11,35	
II	47,88±0,91^{ac}	48,89±1,04^{ac}	49,81±1,21^{ac}	49,34	0,00
III	45,26±0,45^{ab}	16,14±0,81^{ab}	12,82±0,50^{b}	14,48	70,65
IV	44,67±0,84^{ab}	38,42±1,67^{abc}	25,53±1,08^{abc}	33,47	32,16
V	44,96±1,62^{a}	25,03±1,55^{abc}	15,90±0,58^{abc}	20,46	58,52
VI	44,60±0,60^{ab}	21,64±0,92^{abc}	13,85±0,69^{ab}	17,74	64,04

Observation Results of Rat Kidney Organs with Hemactocilin-Eosin Staining

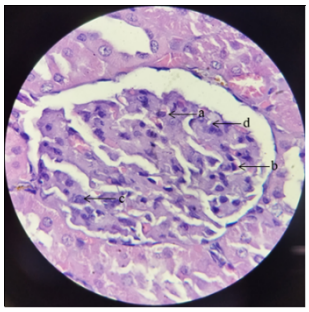
Histopathological observation was carried out to see necrosis in the kidney organs. Necrosis is the death of tissues and cells in living things and is seen in the nucleus. Necrosis due to non-specific breakdown of DNA consists of pyknosis, cariorexis and karyolysis (Kamaliani et al., 2018). In each test group, 2 samples were taken from a total of 30 samples. 1000 times is the magnification used in the observation. Each preparation uses immersion oil to clarify observations.

Under normal circumstances the corpuscle of the kidney is about 200-250 µm in diameter and consists of a tuft of capillaries, the glomerulus, which is surrounded by a double-walled epithelial capsule called Bowman's capsule. Bowman's capsule is called Bowman's space (urinary space) which accommodates fluid filtered through the capillary walls and visceral

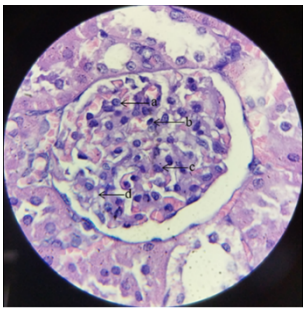
lining. Bowman's capsule on the inside through the visceral layer is composed of modified epithelial cells called podocytes associated with the glomerulus. The connective tissue components of the afferent arterioles do not enter Bowman's capsule and normally the connective tissue cells are replaced by a special cell type, namely mesangial cells (Situmorang & Ilyas, 2018). Observation results and the percentage of kidney organs can be seen in the table and figure below:



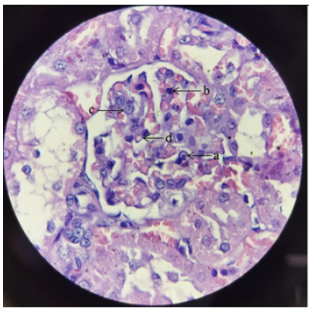
Normal Control



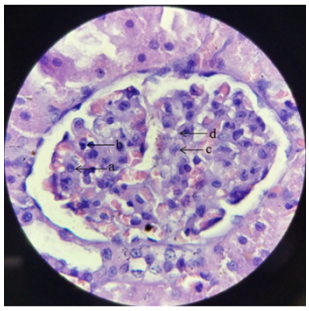
Negatif Control



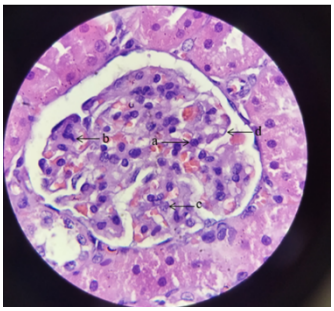
Positif Control



Extract 125 mg/kg



Extract 250 mg/kg



Extract 500 mg/kg

Figure 1. Histopathological Picture Of Rat Kidney

Information :

A. Normal cells

B. Pyknosis

C. cariorexis

D. Kariolysis

Table 6. Calculation of Total Kidney Cell Damage Scoring

Group	Test Animal Code	Types of Cell Damage			Damage Amount	Normal
		Condensation	Karyorrhexis	Kariolis		
	1.1	8	20	3	31	69
I	1.2	11	18	4	33	67
			Total		64±15,63^{bc}	136
	2.2	31	25	8	64	36
II	2.5	29	27	6	62	38
			Total		126±24,57^{ac}	74
	3.3	13	21	7	41	59
III	3.4	15	22	6	43	57
			Total		84±15^{ab}	116
	4.1	25	26	6	57	43
IV	4.2	28	24	7	59	41
			Total		116±22,27^{ac}	84
	5.1	24	24	7	55	45
V	5.4	26	21	6	53	47
			Total		108±20,07^{abc}	92
	6.3	19	21	5	45	55
VI	6.5	18	25	4	47	53
			Total		92±18,90^{ab}	108

Information:

Group I	Normal Control
Group II	Negatif Control
Group III	Positif Control
Group IV	Red betel leaf ethanol extract 125 mg/kg
Group V	Red betel leaf ethanol extract 250 mg/kg
Group VI	Ekstrak etanol daun sirih merah 500 mg/kg
a	Significantly different from the normal control group
b	Significantly different from the negative control group
c	Significantly different from the positive control group

Based on the results of the rat's weight examination, the normal group experienced a continuous increase in weight from day 24 to day 39. The weight gain can be attributed to continuous nutrient intake and the healthy condition of the test animals. In contrast, the negative control group induced with STZ-NA showed a decrease in average body weight from day 24 to day 39. STZ-NA was not given simultaneously, as NA administration was expected to inhibit PARP-1 enzyme activity, thus reducing toxicity and preventing damage caused by STZ to β-pancreatic cells (Saputra et al., 2018; Saputri, 2020). Type 2 DM condition can cause short-term weight loss. This proves that STZ-NA administration has provided hyperglycemia condition with one of its characteristics being weight loss.

Metabolisme yang terganggu terjadi karena berat badan yang turun di akibatkan kekuranngannya insulin (Fitrya et al., 2021). This happens because glucose in the blood cannot enter the cells, causing cells to lack energy. The body will respond by breaking down proteins and fats in the body continuously as an energy reserve, resulting in a decrease in muscle mass and fat tissue, which triggers weight loss (Daeli & Ardiaria, 2018). The test animal's weight loss is due to a decrease in insulin hormone production by β-pancreatic cells, causing glucose not to enter the cells. Insulin deficiency in the test animal results in high levels of glucose in the plasma. If there is not enough insulin in the blood, the cells will begin to starve. Insulin deficiency causes glucose not to be broken down, which means the cells cannot be used (Hediyansah et al., 2019).

The negative group was the treatment group induced with STZ-NA but not given any test preparation, while the positive group given glibenclamide did not experience weight loss due to the mechanism of action of glibenclamide, which triggers the production of insulin hormone by pancreatic β cells so that insulin can work optimally (Katzung, 2012).

From day 31 to day 39, the positive group and the groups treated with ethanol extract of red betel leaf at doses of 125, 250, and 500 mg/kg BW experienced weight gain after initially experiencing weight loss due to the condition of diabetes mellitus as a result of STZ-NA administration. The activity of compounds presents in the ethanol extract of red betel leaf (flavonoids, tannins, saponins, and alkaloids) plays a role in protecting and even regenerating pancreatic β cells, thus triggering the release of insulin. Insulin released will increase glucose transport into cells, including peripheral tissues, thus increasing the utilization of other important nutrients, amino acid absorption, and other macromolecular components (Sudiana & Purwanto, 2019).

The results of post-hoc statistical analysis using Tukey showed significant differences between the normal or non-induced group and the induced group. This indicates that the administration of STZ-NA affects weight loss in rats compared to the normal group.

Based on the results of blood glucose level examination, on the day before STZ-NA induction, the blood glucose levels in all treatment groups, including the normal group, negative group,

positive group, and ethanol extract of red betel leaf groups at doses of 125, 250, and 500 mg/kg BW, were still within normal limits. The normal glucose criterion is in the range of 70-100 mg/dL (S. Ramadhan et al., 2019). On day 24, the blood glucose levels in the negative group increased due to the influence of STZ-NA. Nicotinamide (NA) is an inhibitor of poly ADP-ribose polymerase (PARP) and can inhibit DNA methylation. Administration of nicotinamide before induction with STZ can protect pancreatic cells from the toxic effects of STZ, thus preventing the development of diabetes. NA can partially protect β-pancreatic cells from overall damage. The subsequent administration of STZ has an effect on pancreatic β cells, accompanied by changes in the characteristics of blood insulin and glucose concentrations, leading to hyperglycemia and a decrease in insulin levels in the blood. STZ enters pancreatic β cells through the glucose transporter GLUT2, causing a decrease in the expression of GLUT2. This results in a decrease in peripheral insulin receptor sensitivity, leading to increased insulin resistance and increased blood glucose levels (Saputra et al., 2018).

In this study, an oral anti-hyperglycemic drug was used as a comparator in the positive group. The anti-hyperglycemic drug used was glibenclamide, a sulfonylurea compound that is used as an oral antidiabetic and is a treatment option for diabetes mellitus in patients with hyperglycemia. The selection of glibenclamide as the comparator anti-hyperglycemic drug was considered appropriate because its mechanism of action in the body is by stimulating insulin production from pancreatic β cells and increasing insulin secretion from pancreatic β cells, thus producing enough insulin to regulate blood sugar levels (Karmilah, 2018).

The measurement of glucose levels on day 31 (T3) already showed a decrease in glucose levels in the DM rat group treated with glibenclamide (positive control) and the treatment group using the test formulation. The decrease in blood glucose levels in each treatment group showed that the treatment given in this study had a significant effect compared to the negative group. The most significant decrease in glucose levels occurred on day 39 (T4). The results showed a significant difference ($p<0.05$) between the negative control and the positive control, and the red betel leaf ethanol extract at 500 mg/kg BW, while the red betel leaf ethanol extract at 125 and 250 mg/kg BW were significantly different ($p<0.05$) from both the negative and positive control groups. This indicates that the red betel leaf ethanol extract at 500 mg/kg BW has the same effect as the positive control in reducing blood glucose levels.

This is thought to be due to the flavonoids contained in the red betel leaf. The increase in insulin hormone secretion is caused by the stimulating effect of flavonoids on the sympathetic nervous system (sympathomimetic). Flavonoids increase the intracellular Ca2+ ion concentration and inhibit KATP (ATP-sensitive potassium channel) channels in the Langerhans islets of the pancreas. The closure of these channels will trigger depolarization and open Ca2+ channels, thus increasing the concentration of Ca2+ ions itself and triggering insulin secretion (Ramadhan et al., 2019).

The effectiveness of the red betel leaf ethanol extract as an anti-hyperglycemic can also be seen from the AUC (area under the curve) value, which is used to describe glucose levels at a specific point in time. The AUC of blood glucose levels in rats started on day 0 and continued until day 39 before and after being given the test formulation.

The results of the examination of blood glucose levels showed that the greater the effectiveness of the anti-hyperglycemic power, the smaller the value of the change in the area under the curve (AUC) from 24 to 39 hours, or conversely, the greater the value of AUC from 24 to 39 hours, the lower the anti-hyperglycemic ability provided. In the examination of blood glucose levels, it was found that the normal group had the lowest AUC from 24 to 39 hours compared to the other groups. The values of AUC from the smallest to the largest were in order: normal group, positive group, red betel leaf ethanol extract group at doses of 500, 250, 125 mg/kg BW, and negative group. This means that red betel leaf ethanol extract has anti-hyperglycemic properties

and at a dose of 500 mg/kg BW, it has the same activity as the group given glibenclamide. This indicates that the compounds contained in red betel leaf ethanol extract have the ability to lower blood glucose levels.

Based on the results of blood urea nitrogen (BUN) examination, measurements on day 24 were performed to observe BUN levels after rats experienced diabetic nephropathy complications. BUN levels increased in each treatment group except for the normal group, which did not receive any treatment. The results of statistical analysis using One Way Anova showed that there were differences in each treatment group with significant values ($P < 0.05$). To see the difference in the increase in BUN levels in each group, post hoc tests using Tukey were performed.

Increased BUN levels are caused by impaired kidney function. Type II DM condition activates protein kinase C (PKC) through diacylglycerol. Activated Protein Kinase C then stimulates the activity of angiotensin work, causing glomerular filtration rate (GFR) disturbances. Insulin growth factor-1 (IGF-1), VEGF, and endothelin-1 (ET-1) trigger renal tubule hypertrophy. Protein Kinase C can also stimulate TNF-a and NFκB and increase fibrotic activities such as CTGF and TGF-ß. These two fibrosis factors will increase connective tissue proliferation, causing glomerular hypertrophy and mesangial expansion. A decrease in antioxidant enzyme function is also exacerbated by Protein Kinase C, which stimulates the release of inflammatory cytokines that can cause glomerulosclerosis. The glomerulus and tubules are damaged, resulting in excessive protein excretion (albuminuria and proteinuria) and an increase in urea and creatinine in the blood, which ultimately leads to kidney failure (Sartika et al., 2018).

The increase in BUN levels is caused by decreased kidney function, which is the site of filtration, resulting in the accumulation of BUN in the blood. Impaired kidney function causes a decrease in the glomerular filtration rate and disrupts the excretion of urea, as BUN levels reflect the balance between protein catabolism and urea formation and excretion by the kidneys. BUN measurement results provide an overview of kidney function. The increased BUN level that should have been excreted through urine re-enters the bloodstream, leading to kidney dysfunction. After the test animals experienced type II DM characterized by an increase in BUN levels, each group of test animals was given treatment with the test preparation for 14 days, except for the normal and negative groups (Xie et al., 2019; Xie et al., 2018).

BUN measurements were again taken on days 31 and 39 after the test preparation was given. The results of the BUN measurement in each rat in each group showed a decrease in BUN levels in each group receiving treatment with the test preparation, except for the normal and negative groups. The statistical analysis using One Way Anova showed a significant value ($P<0.05$). To determine the difference in the decrease in BUN levels in each group, post hoc testing using Tukey was conducted. The analysis showed that the best and closest decrease in BUN levels to the normal group was given by the positive control group treated with glibenclamide. Based on the results of the test preparation, the administration of 500 mg/kg body weight of red betel leaf ethanol extract in rats could reduce BUN levels, approaching the decrease in BUN levels in the positive control group.

The decrease in BUN levels in the positive control group is not directly caused by the administration of glibenclamide. Hyperglycemia did not occur because glibenclamide works by stimulating insulin release, which, if it occurs for a long time, will cause filtration disorders in the glomerulus. The decrease in BUN levels produced by the test preparation group was due to the presence of flavonoid compounds. Flavonoid compounds found in red betel leaf can reduce BUN levels by showing a protective effect on diabetic kidneys by reducing oxidative stress. This is because oxidative stress plays a crucial role in the development of diabetic nephropathy complications. Flavonoid compounds can cause an improvement effect on diabetic nephropathy by providing a protective effect on rat kidneys. Saponin compounds can

also reduce BUN levels through hypoglycemic action, regenerate insulin function, provide insulin signaling, release insulin from beta-cell islets, inhibit disaccharide activity, activate glycogen synthesis, inhibit gluconeogenesis activity, inhibit mRNA expression from glycogen phosphorylase and glucose 6-phosphatase, and increase GLUT4 expression (Roosita, 2020).

In addition, based on the observation of rat kidney organs with Hematoxylin-Eosin staining, histopathological profile of kidney with Hematoxylin-Eosin staining was also conducted in this study by reading the damages in the kidney through observing pyknosis, karyorrhexis, and karyolysis. After reading the damages, scoring was conducted to determine the level of damages in each treatment group. Based on the observations, the control group, negative control group, positive control group, and the groups treated with 125 mg/kg, 250 mg/kg, and 500 mg/kg red betel leaf ethanol extract experienced glomerular degeneration and necrosis indicated by pyknosis, karyorrhexis, and karyolysis. The necrosis that occurred in the tubules includes loss of epithelial cells, detachment of the basement membrane, pyknotic intisels, shattered nucleus (karyorrhexis), missing nucleus (karyolysis), and desquamation or loss of a cluster of epithelial cells due to the absence of surrounding tissue that holds it.

From the observation of kidney organ microscopic results and the number of cell damages (pyknosis, karyorrhexis, and karyolysis), the negative control group had the highest number of damages. Kidney cell damage that occurred in the negative control group was caused by the induction of STZ-NA in each test animal which then experienced DM and was left for three weeks, as were the other treatment groups except for the normal control group. In the condition of DM, the glucose level increased as insulin secretion and function decreased. Long-term hyperglycemia conditions lead to continuing damage to kidney function. Uncontrolled hyperglycemia triggers hyperfiltration, which continuously causes nephron damage and becomes sclerosis. The subsequent sclerosis causes angiopathy, which narrows and blocks blood vessels, including those that lead to the kidneys. Thick blood plasma in rats with DM causes slow blood flow. This results in reduced oxygen and nutrient delivery to the tissue, leading to necrosis (eka., 2019). The statistical analysis using One Way Anova showed a significant difference in the number of kidney cell damages in each treatment group ($P>0.05$).

The damage that occurred in the normal control group was because all normal cells physiologically undergo apoptosis. The improvement effect was seen in the positive control group with a total score of 84 for cell damage. The tubule structure improved towards the normal group with epithelial cells still attached to the basement membrane. Glibenclamide does not directly work in repairing kidney function. However, it works by lowering blood glucose levels by stimulating insulin secretion. Damage to kidney function can recover to normal if the cause of the damage is eliminated (Melia et al., 2020). In the test groups, the best improvement effect was shown by the group treated with 500 mg/kg of red betel leaf ethanol extract. The damage that occurred in this group was less compared to other test groups and closer to the total score of cell damage in the positive control group, which was 92. The tubule structure was observed to be better, and the number of cell damages was lower compared to the negative control group and other test groups. Therefore, based on the kidney damage scoring results, the group treated with 500 mg/kg of red betel leaf ethanol extract showed the best activity in regenerating kidney cells. Other groups that showed better results were the normal control group and the positive control group.

The flavonoid compounds contained in the ethanol extract of red betel leaves indirectly play a role in minimizing tissue damage by reducing lipid peroxidation. Flavonoids significantly contribute to the regeneration of damaged pancreatic β cells, thereby overcoming insulin deficiency. In addition, flavonoids act as strong antioxidants which can inhibit cell damage/death (apoptosis) and damage kidney function (Melia-Arisanti, 2020). Flavonoids are broken down into smaller monomeric units when they enter the stomach. Flavonol monomers undergo wider metabolism through biotransformation initiated in erythrocytes and carried by

enzymes originating from the liver and kidney. Once in the small intestine, monomeric units will be absorbed in the form of O-methylated glucuronides, O-methylated and aglycone, which will then enter the portal vein. Flavonoids will undergo metabolism again and be transformed into O-methylated, sulphates, and glucuronides forms. O-methylated enters the cell and functions to counteract apoptotic cell death induced by hydrogen peroxide. The ability of O-methylated to protect cells is related to its ability to donate hydrogen atoms. This fact links flavonoid function in protecting cell death due to oxidant induction through independent antioxidant mechanisms (Sudiana & Purwanto, 2019).

Conclusion

Ethanol extract of red betel leaf at doses of 125 mg/kg BW, 250 mg/kg BW, 500 mg/kg BW can reduce blood glucose levels in STZ-NA-induced diabetic nephropathy rats. The ethanol extract of red betel leaf at a dose of 500 mg/kg BW did not differ significantly from the positive control. The administration of the ethanol extract of red betel leaf at a dose of 500 mg/kg BW provided the most optimal effect on improving kidney function in STZ-NA-induced diabetic nephropathy rats and did not differ significantly from the positive control. The ethanol extract of red betel leaf at doses of 125 mg/kg BW, 250 mg/kg BW, 500 mg/kg BW can improve kidney function by increasing serum albumin in STZ-NA-induced diabetic nephropathy rats. The ethanol extract of red betel leaf at a dose of 500 mg/kg BW did not differ significantly from the positive control. The ethanol extract of red betel leaf at doses of 250 mg/kg BW and 500 mg/kg BW can reduce the amount of cell damage in the kidney organs of STZ-NA-induced diabetic nephropathy rats. The ethanol extract of red betel leaf at a dose of 500 mg/kg BW did not differ significantly from the positive control.

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