Effects of *Ruellia tuberosa* L. Root Extracts on the Pancreatics of Diabetic Rat

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ABSTRACT

Ruellia tuberosa L. is a plant containing triterpenoids from the class of antioxidant and anti-inflammatory flavonoids. This study used four groups of rat: negative control, positive control, 250 mg/kg-bw therapy group and 500 mg/kg-bw therapy group. This study is based on changes MDA levels, blood glucose, histologic of pancreas and changes Insulin levels. Statistical analysis of MDA and blood glucose ($\alpha = 0.05$) showed significant decreases. These results were close to normal which showed by the decreases MDA successively in the therapy group of 27.27% and 67.53% and blood glucose levels of therapy group by 52.95% and 64.24% and the increase of insulin levels in the group therapy respectively 13.25% and 22.69%. Based on the results of histologic observations of the pancreas descriptively showed a decrease in cell damage on Langerhans island. Thus, the provision of root extract of *Ruellia tuberosa* L. can inhibit the damage in the rat DMT1 model.

Key word: *Ruellia tuberosa* L, Malondialdehyde, Diabes Mellitus, Flavonoids.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by the body's inability to metabolize carbohydrates, fats and proteins thereby causing chronic hyperglycemia (elevated blood sugar levels) [1]. Uncontrolled disease will cause various complications of metabolism, macrovascular and microvascular disorders that lead to decrease quality and life expectancy of patients [2]. Statistical reports from the International Diabetes Federation [3] reported that diabetes mellitus every year is increasing where 1 in 11 people have diabetes. The accumulation of diabetics in 2015 is 415 million, and it is estimated that by 2040 it will increase to 642 million people. The number of people with diabetes mellitus in Indonesia on average increased by 2% in each region so that the prevalence of diabetics is estimated to reach 21.3 million in 2030 [4].

The condition of chronic hyperglycemia caused by diabetes is associated with long-term damage, impaired function, and failure of various organs especially the eyes, kidneys, nerves, heart and blood vessels. In diabetics, the cause of damage to pancreatic beta cells can be caused by many factors. These factors include genetic factors, infection by germs, nutritional factors, diabetogenic substances, and free radicals (oxidative stress). Damage to pancreatic beta cells causes the body not to produce insulin, causing blood glucose levels to rise (hyperglycemia occurs). The condition of hyperglycaemia [5] may result in the formation of reactive oxygen species (ROS). Excessive ROS can cause oxidative stress and can

aggravate the destruction of pancreatic beta cells. Oxidative stress occurs due to an imbalance between free radicals and antioxidants, when reacting with the fatty acid component of the cell membrane results in fat peroxidation which will lead to the breakdown of fatty acid chains into various toxic compounds and cause damage to the pancreatic cell membranes that produce malondialdehyde (MDA) [6].

Ruellia tuberosa L. is a plant indigenously found in Indonesia, some paper report secondary metabolites contained its root. For example flavonoids, glycosides, phenolic saponin, carotenoid, steroid and triterpenoid [8] [9]. It was also reported able to reduce lipid levels in model rat [10] and lipid peroxide [11].

This paper reports the effect of root's extract in diabetic model rat. The model was prepared by induction with streptozotocin (STZ). Several studies of STZ induction with treatment Extract of Glycine max (L.) Merr. [7] and effect of Juice Mangosteen Rind (Garcinia Mangostana L.) [8] showed improvement in MDA levels and pancreatic histopathology.

EXPERIMENT
Chemicals and instrumentation

This experimental animals used in the study were white male rats *Rattus novergicus* strain aged 2 months with average body weights (bw) of 180-200 gram purchased from the Animal Model Unit Development (UPHP) Bandung. All animal have been approved by the ethical acceptance of Brawijaya University Research Commision 744-KEP-UB. They have been feed with standard feed and water.

The chemicals used in this study include *Ruellia tuberosa* L. (Material Medika), streptozotocin (Bioworld), n-hexane (Merck), sodium chloride 0.9% (Merck), paraformaldehyde (Sigma Aldrich), azide-phosphate buffer saline (Merck), 90% ethanol (Merck), distilled water (Hydrobatt).

The instrumentation used in this research include rat cages and wire enclosures, masks, drinkers, gloves, Eppendorf centrifuges (OneMed), a set of glassware (IWAKI), incubator, visible spectrophotometer (Thermo Scientific Genesys 20), water baths, 1000 micropipette (Biohit Proline), blue tip (oneMed), Eppendorf tube (biomed), vortex (thermoline), Scissors, spatula, surgical tool and table, vacutainer Non-EDTA (Vaculab), urine pot, mortar and pestle, freezer -20 ^{o}C, 3 mL syringe (Terumo), cuvette, blood lancet (oneMed) and set of Easy Touch GCU.

Preparation of root extract

Extract preparation was undertaken following procedure from Kumar [9]. The root powder obtained was then macerated with n-hexane solvent as much as 7.5 times the weight of the powder. The maceration solution was stirred every 1 h in the first 5 hours and then sterilized for up to 48 hours. The liquid extract obtained was decanted to separate from the powder. The liquid extract was evaporated with a rotary evaporator at a temperature of 50°C, 90 rpm to obtain a viscous extract.

Acclimatization of animal

Groups of rats were placed in a polyethylene cage that filled with wood husks with a dimension of 45 x 35 x 20 cm with wire enclosures. The room temperature was 22 ± 2^oC. the rats were acclimated for two weeks before the experiments. All conditions and handling animals were conducted with protocols approved by Ethical Clearances Committee of Brawijaya University (744-KEP-UB)

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Induction of Streptozoticin

Rats were divided into 4 groups : (1) negative control group, (2) positive control group, (3) therapy of *Ruellia tuberosa* L. with 250 mg/kg of weight dose and (4) therapy of *Ruellia tuberosa* L. with 500 mg/kg of weight dose. These were acclimatized for 2 weeks and injected intraperitonial with streptozotocin solution (20 mg/kg bw) for 5 consecutive days. The animals were incubated for 14 days and analyzed their blood's glucose levels. The diabetic rats have glucose level above 200 mg/dL. 2 group was treated with root extract 2 mL for 21 days. The positive control group (2) was not treated with root extract, and negative control (1) was healthy rats.

Serum collection from blood

After blood coagulated, about 2 hours after collection, blood was centrifuged at 3000 rpm for 15 min, in order to collect blood serum. Serum was moved into Eppendorf tube, and then serum was centrifuged at 1000 rpm for 10 minutes. Serum samples were moved into new Eppendorf tube and stored in -20°C freezer until malondialdehyde levels determination.

Measurement of Malondialdehyde Levels in Serum

Malondialdehyde levels were determined using *thiobarbituric acid* (TBA). The malonreagent levels measurement was performed by adding 100 µL serum wits 550 µL distilled water, 100 µL TCA 20%, 250 µL HCl 1N, 100 µL Na-Thiobarbiturate then incubated for 30 minutes at 100°C. After incubation, sample reading was performed using spectrophotometer UV-Vis at the wavelength of 533 nm.

Histological Analysis of Pancreatic Tissues

Pancreas was fixed in paraformaldehyde solution and was dehydrated with a gradual ethanol series, and then were embedded in paraffin to bring out ultrathin sections of the pancreas. Furthermore, the ultrathin sections were stained with Hematoxylin-Eosin. First, the ultrathin sections were deparaffinized with xylol and rehydrated with a gradual ethanol series (absolute, 95, 90, 80 and 70%) respectively for 5 minutes. Then those were soaked in the distilled water for 5 minutes. Furthermore, the ultrathin sections were dyed with hematoxylin and were incubated for 10 minutes to obtain the best color results. Then the ultrathin sections were washed with flowing water for 30 minutes and rinsed with distilled water. Next, the ultrathin sections were dyed with eosin with alcohol for 5 minutes. The last steps were dehydrated using a gradual series of ethanol (80%, 90%, 95%, and absolute) and cleared with xylol then dried. The dried and ultrathin stained sections were mounted with Entellan and were observed under a microscope with a magnification of 400 times.

Statistical Analysis

The data obtained were analyzed by using Shapiro-Wilk statistic and homogeneity in order to determine the normality of data distribution. Effects of treatment on parameters of total malondialdehyde level were analyzed using ANOVA which was completed by Tukey test with 95% confidence level to know the difference between treatments. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) 23.0 software.

RESULT AND DISCUSSION
Effect of extract *Reullia tuberosa* L.

Root of *Reullia tuberosa* L. previously reported contained some important secondary metabolites, such as triterpenoid [9], saponin [10], and steroid [11], phenolic [12], and

flavonoid [13]. In other research, some of this secondary metabolite has been reported able to reduce glucose level [14]. and has functioned as antioxidative agent [15]. The effect of root extract from *Reullia tuberosa* L on reducing blood glucose level of diabetic model rats and their malondialdehyde level are displayed in Table 1.

Table 1. Profile of MDA, Blood Glucose and Insulin levels in Control, Diabetic, and Therapy

Treatment Group	Blood Glucose Levels (mg/dL)	Average MDA Level (μL/mL)	Insulin Levels (mIu/l)
Control	83.17 ± 6.82	0.164 ± 0.047	9.853 ± 0.38
Type 1 DM	299.67 ± 22.54	0.231 ± 0.02	7.607 ± 0.45
Therapy 250 mg/kg bw	141 ± 24.81	0.168 ± 0.039	8.615 ± 0.25
Therapy 500 mg/kg bw	107.17 ± 10.73	0.075 ± 0.006	9.333 ± 0.2

The levels of MDA in Type 1 DM group were significantly higher compared to non-type 1 DM group. Therapy with *Ruellia tuberosa* L. root extracts may reduce elevated levels of MDA. MDA levels declined with increasing doses of *Ruellia tuberosa* L. root extracts used. Statistical test results showed that there were significant differences ($P<0.05$) between MDA levels of Type 1 DM group and non-type 1 DM group. It suggests that the extracts of *Ruellia tuberosa* L. able to act as an antioxidant, especially as a hydroxyl radical scavenger. The decline in MDA levels related to the decreasing of lipid peroxidation in cell membranes that leads to the reducing of cell membrane damage and inhibition of diabetes mellitus complications. In the treatment group of 250 mg/kg bw and 500 mg/kg bw it can be seen that MDA levels are much smaller than MDA levels in the Type 1 DM group, this is because the formation of free radicals can be suppressed. Therapy group of 250 mg/kg bw decreases MDA levels to 28.97 % and therapy 500 mg/kg bw decreases to 68.74 % of control.

The decrease of insulin content in experimental animal serum was decreased by 22.79%. The decrease in insulin levels in the blood is due to the presence of diabetogenic agents (MLD-SZT) that are induced in animal experiments so that pancreatic beta cells are damaged over time. Streptozotocin can affect pancreatic beta cells because it produces free radicals in the form of hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-).

The experimental group of pletal root extracts with a dose of 250 mg/kg body weight showed a 13.25% increase in insulin level in blood serum indicating that R. *tuberosa* L. root extract was able to inhibit the effect of STZ on pancreatic beta cells, although only slightly. Then in the experimental animal group with a dose of 500 mg/kg of body weight showed an increase in serum insulin levels almost close to the insulin level of the group of negative control animals with an increase of 22.69%.

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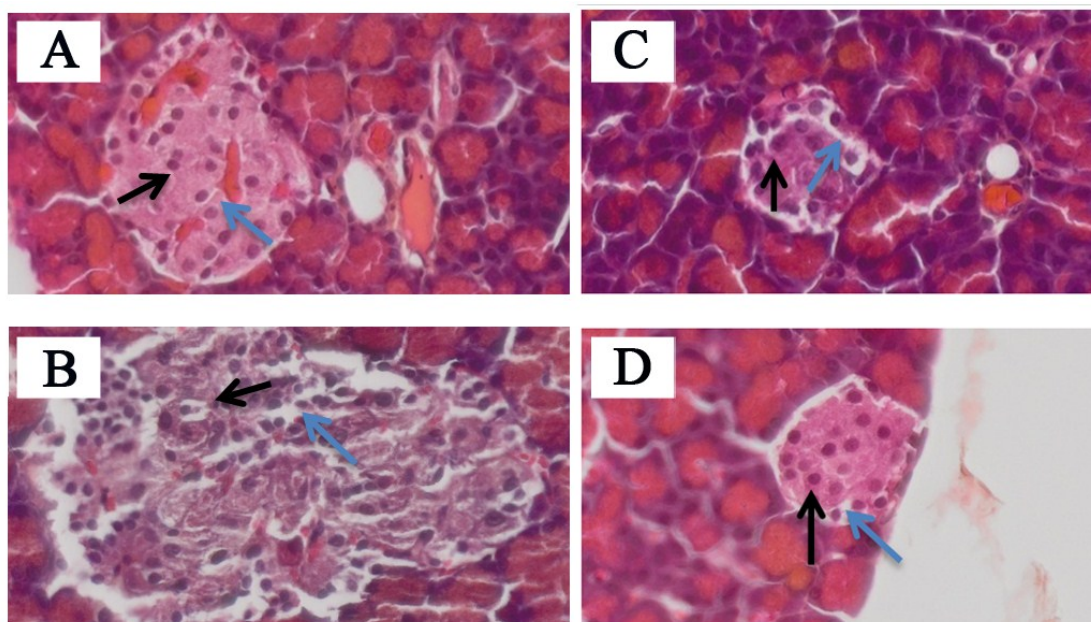
Figure 1. Normal Pancreatic Histology (A) magnified 400 times (Control rat (A), diabetic rat (B) therapeutic rat treated with doses of root extract *R. tuberosa* L. 250 mg/kg bw (C), and 500 mg/kg bw (D). (Cell nucleus (blue arrow), widening the cavities (cellular spaces) (black arrow)).

Changes in pancreatic endocrine cells can be seen through pancreatic histopathologic preparations stained with hematoxylin Eosin (HE) staining. Figure 1 shows a change of pancreatic histology in the streptozotocin-induced group of rats compared to the treatment group of 250 mg/kg bw and treatment group of 500 mg/kg bw of *Reullia tuberosa* L. extract. the histologic changes of the pancreas can be seen from smaller endocrine cells even begin to disappear so that only the empty cytoplasm is visible. In addition, the size of the island of Langerhans on preparations of group B has a smaller size compared to the island of Langerhans on preparations of groups A, C and D. In preparatory group B is also seen the morphology of the pancreas organ that there are gaps or cavities in both endocrine areas (the island Langerhans) as well as in the exocrine region (acinar cells) that indicate a degeneration process.

Langerhans Island in negative controlled animals showed more pancreatic cells, especially pancreatic beta cells than in positive controlled animals, where there was no widening of cavities (intercellular space) on Langerhans island. The widening of the cavity in the pancreatic histopathological picture in the positive control group was due to the severe necrosis that the cell nucleus suffered from death resulting in a shift.

According to Fransiska [16] the changes in cells caused by substances that have a cytotoxic effect are the reduction of the Langerhans islands, the reduction in the number of β cells and degranulation, the vacuolization of these cells. In people with diabetes mellitus, some β cells show complete degranulation and an empty cytoplasm.

The results of observations of pancreatic histopathologic preparations from experimental rat animals given *Ruellia tuberosa* L. extract showed better Langerhans island than the pancreatic group of Positive Control (B) rat (Figure 1). Changes that occur include the number of β cells are more, β cells spread throughout the island Langerhans, larger island size Langerhans and cavities in the endocrine region of the pancreas fewer than the pancreas

in the KP group. This change shows the endocrine cells that begin to regenerate into normal shape.

This is due to the treatment of *Ruellia tuberosa* L. extract which can inhibit the damage of β cells. Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals to cells. Antioxidants stabilize free radicals by supplementing electron deficiencies possessing free radicals and inhibiting the occurrence of chain reactions from the free radical formation which can cause oxidative stress.

CONCLUSION

It can be concluded from this study, Therapy of root extract of *Ruellia tuberosa* L. with varieties of doses (250 and 500 mg/kg bw) in diabetic rats which is induced by MLD-STZ showed a decreasing of MDA level, increasing of Insulin levels and a repair of histology of pancreatic tissues in accordance with the increasing dose given.

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