The Effects of Root Extract *Ruellia tuberosa* L on Histopathology and Malondialdehyde Levels on the Liver of Diabetic Rats

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Abstract. The aim of this research is to study antidiabetic activity of root extract of *Ruellia tuberosa* L on rats (*Rattus norvegicus*) induced by multiple-low dose streptozotocin as animal diabetic models. The parameters investigated were blood glucose levels, free radicals (MDA, malondialdehyde) levels and hepatic histopathology. The main materials used were n-hexane root extracts from *Ruellia tuberosa* L. Three groups of rats, including control group (group I), diabetic group (group II), and therapy group with *Ruellia tuberosa* L (group III), were used. Streptozotocin was given at multiple-low dose of 20 mg/kg of body weight for 5 times in 5 consecutive days *i.p.* to rats in groups II and III. The *Ruellia tuberosa* L extracts were then given orally for group III in the dose of 250 mg/kg of body weight per day for 3 weeks. Results of the current work showed that root extract *Ruellia tuberosa* L had lowered blood glucose levels on rats in group III by 60.3%, from 299.7 ± 24.7 mg/dL up to 119.0 ± 26.6 mg/dL. Moreover, the antidiabetic activity of *Ruellia tuberosa* L extracts also deduced from decrease of MDA levels in group III, from 3.5 ± 0.3 μg/mL up to 1.7 ± 0.4 μg/mL. The recovery of hepatic organ from treatment group has also been proven from the its histology profiles stained with hematoxylin-eosin.

1. Introduction

Diabetes mellitus type 1 is a metabolic disorder caused by the destruction of pancreatic β cells, resulted in reduced production of insulin hormones, that causes hyperglycemia [1]. Prevalence of diabetes worldwide is estimated at 171 million people in 2000, and is expected to increase to 366 million by 2030 [2]. In Indonesia, the number of people with diabetes mellitus reached 14 million in 2006, and is expected to reach 21.3 million people in 2030 [3].

Hyperglycemia in diabetes mellitus closely related with oxidative stress conditions [4] and increase of ROS (reactive oxygen species) production. Increasing ROS causes an increase in lipid peroxidation. ROS will degrade PUFA resulting in malondialdehyde compound [5]. Malondialdehyde compounds are one of the signs of lipid peroxidation due to excess ROS. The higher the amount of ROS in the body the higher the levels of malondialdehyde [6]. In addition, the presence of toxic substances in the body will cause damage to liver cells. The liver is an organ that plays role in the toxic detoxification process that enters the body thus it can easily be damaged. When the liver organ is constantly exposed to toxic substances, this will result in degeneration of liver function that causes damages to the liver cells. Damage to liver cells will be easily observed through microscopic histopathological observations.
Treatment of diabetes mellitus can be conducted with insulin or by administration of antidiabetic drugs such as sulphonylurea and biguanid derivatives. However, such treatments can cause very serious side effects such as hypoglycemia, obesity, and renal dysfunction [8, 9]. In order to overcome these problems, it is necessary to search for alternative treatment from indigenous natural products from Indonesia. One of the plants that commonly used for diseases treatment is pletekan plants from the species of *Ruellia tuberosa* L. This plant has been proposed to have potency as anti-diuretic, antidiabetic, antipyretic, analgesic, antihypertensive, and antidotal agents [10]. Manikandan (2010) in his research reported that leaf of pletekan contains phenol compounds (0.36 mg/g), saponins (0.10 mg/g), glycosides (0.59 mg/g), flavonoids (0.75 mg/g) and vitamin (K, C) and carotenoid content [10]. The bioactive compounds of pletekan root obtained from GC-MS revealed 25 compounds including steroid form of stigmasterol 8.89%, sitosterol 3.99%, cholesterol 2.24% and triterpenoid form of Lupeol by 68, 14% [11]. Triterpenoid is the major bioactive compounds contained in the root pletekan can act as antioxidants [11], which can decrease ROS [12, 13].

The part of pletekan that already widely utilized to overcome hyperglycemia are leaves and stems. Rahmi et al studied of hypoglycemic effects purple gold leaf extract (*Ruellia tuberosa* L.) on Wistar rat mentioned that leaf water extract of pletekan can be lowered glucose levels in rat blood [14]. Utilization root pletekan actually already exists but still traditional and there has been no scientific research on the effects of hypoglycemia. Based on the description above, this study has been conducted to determine the effect of root pletekan root to decrease the malondialdehyde level and improve the histopathology on the liver of diabetic rat induced by MLD-STZ.

### 2. Materials and Methods

#### 2.1 Materials

This research used the animal model of male Wistar white rat obtained from Institute of Biosciences Brawijaya University, Malang. The experimental animals have received a certificate of ethics from Research Committee of Universitas Brawijaya No: 624-KEP-UB-2016. The materials used in this research were root of *Ruellia tuberosa* L. obtained from Materia Medica, Batu City, East Java. Other materials used were distilled water, conc. H2SO4, citric acid solution, sodium citrate solution, citrate buffer solution pH 4.5, streptozotocin (Bioworld), PBS solution (Phosphate Buffer Saline), 1% PBS-azide solution, 10% trichloroacetic acid (TCA), HCl, 1% Na-thio, NaOH solution, ethanol, xylol, paraffin, hematoxyline eosin (HE), and 10% paraformaldehyde (PFA).

#### 2.2 Methods

##### 2.2.1 Preparation of Diabetic Rats

A week prior to the experiment, rats were adapted in the animal house. Rats were divided into three groups: (I) control group; (II) diabetic group; and (III) treatment group, 6 rats in each group. Rats were maintained in the animal house, Biosains Laboratory, Brawijaya University. A 100 mg of streptozotocin 100 was dissolved in 3 mL of citrate buffer at pH 4.5. A dose of 20 mg/kg of body weight was injected *i. p.* to rats in groups II and III, for 5 consecutive days, while rats in group I (control) were injected with PBS only [15-17]. Rats in group III were treated with root extracts at a dose of 250 mg/kg body weight per day for each rat for 21 consecutive days.

##### 2.2.2 Extraction of Pletekan Root

Root powder was macerated with n-hexane, the volume was 7.5× the weight of the powder. Maceration solution was stirred every 1 h in the first 5 h, and then allowed to stand for up to 48 h. The extract obtained was decanted to separate from pletekan powder. Solvent in the extract was evaporated with a rotary evaporator at 400 °C, 90 rpm, until the concentrated extract obtained.

##### 2.2.3 Treatment with Root Extracts of Pletekan for Group III

Rats in group III were treated with pletekan root extracts at a dose of 250 mg/kg body weight per day for each rat for 21 consecutive days. Changes in blood glucose levels were monitored per week for during the treatment. At the end of the assay, rats were sacrificed, and the hepatic organ was collected for further analysis.
2.2.4 Measurement of Malondialdehyde (MDA) Levels
Measurement of MDA levels was conducted by spectrophotometry method, using TBA reagents. The liver homogenates from three groups I, II, and III were prepared based on previous method [18]. The liver supernatants were measured their absorbance using a UV-Vis spectrophotometer at the 530 nm.

2.2.5 Liver Histopathological observation
Histopathologic preparations of the rats’ liver were made using the HE staining method. Histopathologic features of the liver were visualized using the Olympus BX51 microscope with 400× magnification to observe the degeneration of lipid and damage to hepatocyte cells. Hepatic histopathology images were captured using a digital camera.

2.2.6 Statistical Analysis
The Student’s t-test was used for statistical analysis of differences between two groups of samples, control and treatment, or between each treatment. In all cases, any result with a p threshold < 0.05 was considered to be significant.

3 Results and Discussion
Injection of streptozotocin with a dose of 20 mg/kg body weight 5 consecutive days caused rats suffered from DM type 1. Table 1 presents the blood glucose levels on rats in all groups in the last week of the assay (week 3).

Table 1. Profiles of blood glucose levels on rats from group I, II, and III, after 21 d of treatment with pletekan root extract

| Group | Blood glucose levels (mg/dL)* |
|-------|-------------------------------|
| I     | 83.17±7.46a                   |
| II    | 299.67±24.71b                 |
| III   | 119.3±24.63c                  |

*different notations show a significantly different effect in each group (p <0.05)

Based on the data on Table 1, it can be seen that blood glucose levels in group I was at a range of normal rats, 83.17 ± 7.468 mg/dL, while blood glucose levels in rats injected by MLD-STZ were 299.67 ± 24.695 mg/dL. These show an increase of 260.31% when compared with control rats. The treatment of pletekan root extract with the dose of 250 mg/kg body weight decreased blood glucose level to 119 ± 24.633 mg/dL, with a percentage of decrease was 60.29%. Decreased blood glucose levels in the treatment group is suggested as a result of improvement of pancreatic β cells, hence, insulin secretion into the bloodstream will improve the process of glucose uptake.

Next is determination of root pletekan extract on the hepatic MDA levels. Malondialdehyde is one of the end products of cell membrane lipid peroxidation by excess ROS, therefore, MDA is used as one of the markers of ROS levels in the body [16, 19]. Measurement of MDA levels (Table 2) shows that MDA levels in diabetic rats group were 3.48 ± 0.27 μg/mL, this value increased 136.58% when compared with the negative control group with MDA levels at 1.47 ± 0.32 μg/mL. In the treatment group MDA level was 1.69± 0.37, or decreased by 51.55%.
Table 2. MDA levels on rats from group I, II, and III, after 21 d of treatment with pletekan root extract

| Group | MDA levels (µg/dL)* |
|-------|---------------------|
| I     | 1.47±0.32a          |
| II    | 3.48±0.27b          |
| III   | 1.69±0.37a          |

*different notations show a significantly different effect in each group (p <0.05)

Figure 1. Comparison of histopathologic images of rats from group I, II, and III, after 21 d of treatment with pletekan root extract, with HE staining (400× magnification); (A) group I; (B) group II; (C) group III, black arrow(↑) indicates the hepatocyte cells, the blue arrow(↓) indicates a sinusoid.

Intraperitoneal injection of MLD-STZ resulted in toxic substances circulated into the body and going directly into the circulatory system. The liver plays a role in the detoxification process of toxic substances in the body is very susceptible to damage. The liver cells that are continuously exposed to toxic compounds such as STZ as diabetogenic agents can trigger an increase in the production of excessive free radicals resulting in oxidative stress. Excessive amounts of free radicals will increase the peroxidation of cell membrane lipid peroxidation resulting in increased levels of MDA. It is therefore, the MDA levels on the diabetic conditions generally is higher than in the normal state.
MLD-STZ injections and pletekan root extract therapy have an effect on the histopathologic picture of diabetic mouse liver. Changes in the histopathologic picture of the liver can be seen in Figure 1. Histopathologic changes of hepatic features can be seen from hepatocyte cells (hepatic cells). The hepatocyte cell condition of the negative control group shows that normal hepatocyte cells are arranged radial, the cell nucleus is clearly blue, the space between hepatocytes or sinusoids is clear and the absence of fat accumulation in the liver. The hepatocyte cells of diabetic rats show that the cell nucleus were damaged and black. In addition, there is dilation of sinusoids and suppress the cell nucleus to peripheral hepatocytes. Improvement in histopathologic features of the liver can be seen in the treatment group. Histopathologic improvement is evident from the presence of normal hepatocyte cells but still appears to be part of hepatocyte cells whose nuclei are damaged. In addition, the widening of the sinusoid begins to improve, thus, the space between hepatocyte cells is narrower.

Decreases in blood glucose and in MDA levels, improvements in hepatic profiles of diabetic rats treated with pletekan root extract can be associated with the content of triterpenoid in the composition of pletekan root extracts. Preliminary phytochemical test has been conducted, and result indicated that the root extracts of pletekan contained triterpenoid. These were deduced from the color change from orange after to red-purple when the sample solution was tested with Lieberman-Burchard reagent (result not shown). This suggests that triterpenoids in pletekan roots extract begin to suppress the presence of free radicals in order not to bind to the lipid component of PUFA in hepatocyte cells by removing the H atoms in the hydroxyl group (-OH), as a result, the free radicals will bind to atoms with high electronegativity. Moreover, triterpenoids will balance the amount of oxidants and antioxidants in the body that play the role of protecting cells from free radicals.

4. CONCLUSION
The current study demonstrated that administration of pletekan root extract decreased blood glucose level in diabetic rats, decreased malondialdehyde levels, and improved histopathological profiles on the liver. The triterpenoid contained in the pletekan root extracts may be responsible for the antidiabetic actions. Further research is needed to investigate molecular mechanisms of triterpenoid acting as free radical scavengers.

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