The effect of sikkam (*Bischofia javanica*) leaf extract on blood sugar levels and islet of langerhans in alloxan-induced diabetic rats

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Abstract. Diabetes Mellitus is a metabolic disease characterized by hyperglycemia, occurring due to abnormal insulin secretion or insulin action. Alloxan induces diabetes by damaging pancreatic cells and initiating hyperglycemia. One of the plants that has the potential to decrease blood sugar is leaves of sikkam (*Bischofia javanica*), because it contains quercetin and gallic acid compounds which are classified as an antioxidant group. The objectives of this study were (1) to analyze the effect of sikkam leaf extract on the reduction in blood sugar levels of Wistar rats and improved the histology of langerhans islet; and (2) determine the dose of sikkam leaf extract which has the most effective effect in reducing rat blood sugar levels. The design used is a non factorial complete random design. The samples used were 30 Wistar rats which were divided into 6 treatment groups and 5 repetitions, namely K₀ (negative control), K₁ (positive control), K₂ (dose of ethanol extract of sikkam leaf (EESL) 300 mg/Kg BW), K₃ (EESL 600 mg/kg BW), and K₄ (EESL treatment 900 mg/kg kg) and K₅ (Glibenclamid 4.5 mg). Data analysis used One-way ANOVA followed by LSD test. The results showed that there was an EESL effect on the reduction in rat blood sugar levels and improved the histology of Langerhans islet. EESL which has the best antidiabetic effect is on administering a dose of 900 mg/kg BW.

1. Introduction

The use of plants as antidiabetic agents has become a research trend regarding the management of diabetes over the last few decades. That is because treatment using plant extracts is considered safer, relatively inexpensive and several other antidiabetic plants have been laboratory tested to improve the structure of the pancreas which is an insulin factory. According to [1], one of the plants that has the potential as an antidiabetic agent based on its chemical content is the sikkam plant (*Bischofia javanica*).

This plant is found widely in Vietnam, India, Indonesia and Philippines [2]. Sikkam is a plant species from the family Phyllanthaceae which is reported to be rich in flavonoid and phenolic compounds such as quercetin and gallic acid which have high antioxidant activity [3], [4], [5,6]. Sikkam bark has traditionally been used by the Pakpak and Simalungun communities in North Sumatra as an ingredient for the treatment of various types of diseases, including diabetes [7,8]. In India also this plant has been proven to be able to reduce laboratory blood sugar levels of white rats, namely the ethanol extract of the bark [9].
Diabetes mellitus is a disorder of sugar metabolism that occurs because the pancreas does not produce enough insulin or when the body cannot effectively use insulin [10]. Diabetes that is not treated immediately can cause serious complications. The number of cases and the prevalence of diabetes have continued to increase over the past few decades and will continue if not treated seriously [11]. The condition of diabetics who have blood sugar levels above normal limits (hyperglycemia) will be involved in the formation of free radicals because it causes glucose auto-oxidation, protein glycation, and activation of the polyol metabolic pathway which will further accelerate the formation of reactive oxygen compounds or Reactive Oxygen Species (ROS) [12]. The formation of ROS is able to increase the modification of lipids, DNA, and proteins in various tissues which is the beginning of oxidative damage known as oxidative stress. This phenomenon occurs in all cells of the body, including the pancreatic beta cells as insulin factories [13].

According to [14,15], oxidative stress can be neutralized by antioxidant compounds such as flavonoids and phenolics by donating hydrogen atoms. Isolation of sikkam leaf chemical compounds carried out by [1,4,16] discovered the presence of gallic acid and quercetin. Quercetin, which has antioxidant effects, has been shown to prevent pancreatic β cell damage due to oxidative stress, help increase insulin secretion, increase adipokine secretion, inhibit glucosidase activity in the small intestine, and increase GLUT4 transporters in skeletal muscle [17,18]. Whereas gallic acid provides an antidiabetic effect by increasing the use of glucose into energy and increasing insulin sensitivity [19].

Based on library research, no reports have been found on the effects of sikkam (Bischofia javanica) leaf extract on blood sugar levels, body weight and repair pancreatic rat tissue in diabetes of rats due to diabetes. Therefore, this study will examine the effect of ethanol extract of Bischofia javanica leaves on blood sugar levels and body weight of mice induced by alloxan diabetes.

2. Method

2.1. Materials and Tools
The materials used in this study were: sikkam leaf, alloxan, CMC-Na, glibenclamide, filter paper (whatman), 70% ethanol solvent. The tools used in this study: rat cages, gloves, eating and drinking animals, glassware (pyrex), sifter simplicia, scissors, 3 ml disposable syringe, Nasogastric tube (NGT) no. 3,5, analytical scales, rotary vacuum evaporator, oven, blender (Laboratory blender), mortar and mortar, 1 set of blood sugar strip measuring devices (Auto Check), then all of the tools and materials for the H&E coloring process.

After 28 days, rat were dissected and pancreatic tissue was taken. The pancreas of each group of rat was fixed with 10% formal saline for 72 hours, dehydrated with ethanol, cleaned in xylene and planted in paraffin wax. With a thickness of 5 μm, the pancreas is stained with Hematoxylin and Eosin. Through observation of pancreatic tissue that has been colored with the H&E method, data will be obtained in the form of area and diameter of the islet of Langerhans due to changes in the number of endocrine cells, especially beta cells. observations were made with a microscope equipped with Optilab software.

2.2. Preparation of alloxan solution
Alloxan monohydrate corresponding to a dose of 125 mg/kg BW was dissolved in 0.9% w/v NaCl solvent. The dosage used was calculated based on the weight of each rat.

2.3. Preparation of sikkam extract
Ethanolic extraction of sikkam leaves is done by maceration method, which is weighed simplicia as much as 100 g then extracted with 1000 ml of 70% ethanol by maceration for 5 days (every day stirring). The extract was then filtered using whatman filter paper obtained (maserat 1) and the remainder (debris) was extracted again for 2 days with 500 ml of 70% ethanol then filtered using whatman filter paper obtained (maserat 2).
Furthermore maserat 1 and 2 are collected and evaporated with a rotary vacuum evaporator at 40°C, then followed by drying in an oven at 40°C to produce a thick extract. The dosage of extract was based on the toxicity test dose of [5] and modified. Then converted to body weight (BW) of rat with reference to the dose conversion [20]. So these the 3 doses used are 300 mg/kg BW, 600 mg/kg BW, 900 mg/kg BW.

2.4. Preparation of glibenclamide suspension
As much as 4.5 mg of glibenclamid is put into a mortar and crushed then dissolved in distilled water up to 36 ml. After that the glibenclamide solution was given to the test animals according to the weight of each rat.

2.5. Treatment of laboratory animals
Total of 30 Wistar rats (Rattus norvegicus) were divided into 6 treatment groups with 5 replications. Test animals were made in hyperglycemia conditions by giving alloxan 125 mg/Kg BW unless the control group was negative. K0 (negative control), K1 (positive control), K2 (EESL 300 mg/Kg BW), K3 (EESL 600 mg/Kg BW), K4 (EESL 900 mg/Kg BW), K5 (Glibenklamid 4.5 mg). Each treatment was given orally, once every day and tested for 28 days.

2.6. Statistical analysis
Data will be presented in the form of mean ± standard deviation. The difference between area and diameter of the langerhans islet was analyzed using the one-way analysis of variance (ANOVA) test. The post-hoc least significant difference (LSD) test was used to determine the average specific differences of each group if the analysis using the one-way ANOVA test showed significant results. P values <0.05 indicate statistically significant results.

3. Results and Discussion
The results will be discussed in 2 subsections, they are blood sugar levels (BSL) and body weight (BW) measurements.

3.1. Blood sugar levels
Measurement of BSL in each group 5 times, namely before induction of alloxan (M0), after induction of alloxan (BSL into diabetes that is ≥ 200 mg/dL) and then continued to be induced treatment and measured every week for 28 days. Then obtained BSL week 1 (M1), week 2 (M2), week 3 (M3) and week 4 (M4). Observation of BSL can be seen in Figure 1.

![Figure 1. Blood sugar levels of R. norvegicus during treatment](image)
Based on the graphs of BSL showed the different results in each time range from M (0) to M (4) in each group. K0 for the alloxan-induced negative control group shows a constant graph at 100 until the end of measurement M (4). The graph of K1 for the test group as a positive control experienced an increase in every measurement week, BSL reached the highest rate of 450 mg/dl at week 4. Of the three doses of EESL, the decrease in BSL up to week 4 was significantly the K4 group, namely the group with dose of ethanol extract of sikkam leaves (EESL) of 900 mg/Kg BW. This dose shows a decrease in BSL with a high level of similarity with the K5 group induced Glibenclamid as a comparison group, which is in the range of BSL values 110-120 mg/dl.

EESL dose 900 mg/dl as the most effective dose in reducing BSL, has a similar effect to the effect caused by Glibenclamid. This decrease is thought to be caused by regeneration of Langerhans β cells which can still secrete insulin due to the induction of Alloxan which does not damage all pancreatic β cells. This shows that Bischofia javanica leaves have antidiabetic effects. According to the results of the isolation of sikkam leaf chemical compounds conducted by [1,4,16] found the presence of gallic acid and quercetin which is classified as an antioxidant group.

Rats treated with alloxan will experience pancreatic cell damage and cause persistent hyperglycemia. This condition of diabetics who are hyperglycemic will be involved in the formation of free radicals because it causes glucose auto-oxidation, protein glycation, and activation of polyol metabolic pathways which will further accelerate the formation of reactive oxygen compounds or Reactive Oxygen Species (ROS) [12]. The formation of ROS is able to increase the modification of lipids, DNA, and proteins in various tissues which is the beginning of oxidative damage known as oxidative stress. According to [14,15], oxidative stress can be neutralized by antioxidant compounds such as flavonoids and phenolics by donating hydrogen atoms. So sikkam leaf extract in this study has a high effectiveness in reducing BSL due to the presence of antioxidant compounds namely dalat acid and quercetin.

3.2. Body weight measurements
Observations of BW measurements were made using analytical balance sheet. The time of animal weight measurement is carried out simultaneously with the BSL measurement time.

![Figure 2. Body weight of R. norvegicus during treatment](image-url)
Through the graph it can be seen that BSL affects the weight of the test animals. In the negative control (K₀), BW looks normal and constant at 170 g ranging from M₀ to M₄. However, the graph K₁ appears to be the opposite of graph K₀. K₁ has decreased significantly from M₁ to M₂, from 180 g to 140 g. This number is inversely proportional to the BSL value. When BSL rises, the BW will decrease. The graph of BW increase in the case of BSL falling towards normal BSL can be seen on graphs K₄ and K₅. Even though the BW increase figure does not seem significant, BW does not decrease when BSL is at normal values.

After doing the correlation test, the correlation coefficient (R²) is 0.712. Correlation correlation value between 0.61 - 0.80 is classified as sufficient correlation [21], so it can be said that BW and BSL have a relationship. If there is a scientific review of why BSL affects BW, this is because insulin is known as a glucose absorption receptor through a special membrane of insulin sensitive which results in an increase in BSL due to delayed glucose uptake [22] and [23]. But this is not always related due to many factors including adrenaline levels, uncontrolled fasting blood glucose (FBG) diets [24], excessive and genetic food consumption [25].

3.3. Histopathological observation of pancreas

The observations showed that there were differences in the area and diameter of the Langerhans islet of each group.

| Table 1. Morphometry of Langerhans Islet for Each Test Group (Area and Diameter) |
|---------------------------------------------------------------|
| Treatment | Area (µm²) | P  | Diameter (µm) | P  |
| K₀        | 4926,51 ± 107,22 a |  | 81,81 ± 6,62 |  |
| K₁        | 4717,34 ± 115,52 a |  | 77,71 ± 8,4 |  |
| K₂        | 4547,29 ± 122,11 a |  | 75,12 ± 6,01 |  |
| K₃        | 5990,93 ± 135,36 a,b | 0,042 | 86,61 ± 9,77 | 0,013 |
| K₄        | 7630,19 ± 147,59 b,c |  | 98,97 ± 10,44 |  |
| K₅        | 7585,36 ± 142,31 b,c |  | 97,34 ± 9,84 |  |

Description: Different notation shows a significant difference (p≤0.05) with the LSD post-hoc test

One-Way ANOVA test results indicate that the value of sig p (BSL, BW, Area and Diameter are less than 0.05) which means there is a significant difference between the six treatment groups or in other words the population variant is not the same. That mean, the null hypothesis (H₀) is rejected, meaning that there is an influence of B. javanica leaf extract on the reduction of BSL, BW, area and diameter of the rat Langerhans islet.

To see a significant difference in the mean values between each treatment group that experienced a statistical difference, the test continued with the LSD test. LSD test results show a significant or significant difference if the significance of each treatment group is less than 0.05 (≤0.05). The Glibenclamid (K₅) treatment group did not provide a significant difference with the EESL group with a dose of 900 mg / Kg BW. Furthermore the negative control treatment group did not provide a significant difference with EESL (300 mg / kg body weight dose and 600 mg / kg body weight dose). This means that the EESL dose 900 mg / Kg BW has an effect similar to glibenclamide. Thus the EESL dose 900 mg / kg BW has the effect of reducing blood sugar levels, affecting body weight, and increasing the histology of islets of langerhans rats for 28 days. If seen from the comparison of the smallest significance value below 0.05 from the EESL treatment group with the positive control group, the dose of 900 mg / kg BW has a value of 0.04. This means that this dose has the most effective effect on these four parameters statistically compared to the other 2 EESL dose.
**Figure 3.** The morphology of the Langerhans islet; negative control group (A), positive control (B), EESL dose 300 mg / Kg BW (C), EESL dose 600 mg / Kg BW (D), EESL dose 900 mg / Kg BW (E), and Glibenclamid (F) control. H & E. Magnification at 400×
Through the Table 1. It can be concluded that the treatment group with the broadest area of the langerhans islet is the K₄ group. If compared to the K₅ group, the EESL 900 mg / Kg BW treatment was more effective to improve the structure of Langerhans Islet which contained endocrine cells such as Beta cells that play an important role in insulin release. Compared to K₀ group, K₄ group has an average area of Langerhans islet above the area of Langerhans islet K₀ group. In Histology studies, this phenomenon explains that regeneration of Beta cells and other endocrine cells due to EESL administration with the most effective dose is 900 mg / Kg BW (Fig. 3 E). Langerhans Islet can be formed through the proliferation of differentiated endocrine cells[26], undifferentiated stem cells [27] or neogenesis of pancreatic ducts [28]. Neogenesis is the proliferation and differentiation of progenitor cells that determine the number of β cells at birth. A small portion of the β cell cycle can still continue to develop if it is needed as a compensation mechanism for increasing insulin requirements. Factor stimulation externals such as pancreatectomy and treatment can contribute to increasing the regeneration of Langerhans Islet cells [29]. Beta cell degeneration and endocrine cells of the islets of Langerhans are evident in the K₁ group (Figure 3B). Characterized by the formation of cavities in the islet of Langerhans. This cavity formation occurs due to the reduction in endocrine cells in the islet as a result of oxidative damage caused by administration of Aloxan.

Qualitatively and quantitatively research data indicate an improvement in pancreatic tissue although the mechanism of EESL on pancreatic beta cell regeneration is not yet clearly known. Based on previous research, active compounds with antioxidant abilities and free radical scavengers can help regenerate beta cells and protect pancreatic islet cells from the cytotoxic effects of alloxan [30].

4. Conclusions
Based on the results and discussion, the following conclusions can be drawn. 1) There is an effect of Bischofia javanica leaf extract on the BSL reduction of Wistar rats (Rattus norvegicus). This shows that Bischofia javanica leaves have antidiabetic effects or act as antihyperglycemic agents. 2) The dose of Bischofia javanica leaf extract of 900 mg/KG BW has the most effective effect in reducing BSL of Wistar rats (Rattus norvegicus) with an average value of 136.80 mg/dl or not significantly different from negative or normal control treatments with an average of 166.08 mg/dl.

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