Evaluating the Efficacy of *Clerodendrum minahassae* Ethanol Extract on Insulin Regulation in Diabetic Wistar Rats

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**Abstract**

Leilem plant (*Clerodendrum minahassae* Teisjm & Binn.) from the genus Clerodendrum has the potential as antidiabetic, antihypertensive, anti-inflammatory, antioxidant, antimalarial, antitumor, antidiarrheal, antimicrobial and antihyperlipidemic. This study aimed to see the effect of ethanol extract of *Clerodendrum minahassae* (CM) leaves on increasing insulin levels in diabetic Wistar rats induced with streptozotocin. This study was conducted in vivo, using 20 rats as experimental animals. The experimental animals were divided into four groups, namely the negative control group (Na-CMC 0.5%), the ethanol extract group of leilem leaves 250 mg and 500 mg, and the positive control group (glibenclamide) as a comparison. Each experimental animal was induced streptozotocin intraperitoneally; then, each solution was given for 14 days according to the test group. After the treatment, the animals were terminated for blood collection; the blood was then centrifuged to obtain blood plasma serum. Blood plasma serum was measured by the ELISA Kit (Rat/Mouse Insulin) method, and then the results were read on a spectrophotometric device. The results of the sample insulin concentration obtained showed that 250 mg/kgBW and 500 mg/kgBW of the CM ethanol extract group could increase insulin levels in diabetic Wistar rats, the same as the positive control group glibenclamide. In contrast, the Na-CMC 0.5% as a negative control group did not show a significant increase in insulin levels. Leilem leaves can be developed for further research on their antidiabetic activity both in vitro, in vivo, and in silico, as well as their toxicity.

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**1. Introduction**

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels, leading to significant morbidity and mortality rates worldwide [1]. The global prevalence of diabetes is projected to increase dramatically, with estimates suggesting that approximately 642 million individuals will be affected by the year 2040 [2]. Recent data positions Indonesia as the sixth country with the highest number of DM cases among those aged 15 years and older, with a reported prevalence of 10.8% [3]. The increasing prevalence of DM, particularly type 2 diabetes mellitus (T2DM), poses a significant burden on healthcare systems and highlights the need for effective management strategies.

T2DM is the most prevalent form of diabetes, characterized by a progressive decline in insulin secretion in the context of insulin resistance [4, 5]. The management of T2DM encompasses five essential
components: dietary control, physical activity, metabolic status monitoring, pharmacological therapy, and patient education. However, many patients with T2DM face challenges in self-management, including maintaining a healthy diet, engaging in regular exercise, adhering to medication regimens, monitoring blood glucose levels, and managing stress. Furthermore, concerns about out-of-pocket medical expenses contribute to the failure of diabetic patients receiving insulin therapy to achieve therapeutic targets [6].

Although numerous novel synthetic antidiabetic drugs have been developed to manage hyperglycemia in diabetic patients, many of these medications carry the risk of adverse effects [7], limiting the long-term treatment of T2DM using synthetic drugs. In contrast, traditional herbal remedies for diabetes are widely used globally and present a promising alternative for managing T2DM [8]. Plants contain various bioactive compounds, such as carotenoids, flavonoids, terpenoids, alkaloids, and glycosides [9–12], which often exhibit antidiabetic properties [13]. Clerodendrum minahassae (CM), a plant native to Indonesia, has been found to contain phytochemical compounds, including alkaloids, flavonoids, steroids, saponsins, phenols, and tannins, in the ethanolic extract of its leaves [14, 15]. These compounds can potentially exert antidiabetic effects, making CM a promising candidate for further investigation.

Despite the growing interest in herbal remedies for the management of T2DM, there is a lack of research on the anti-diabetic properties of CM. To address this gap, the present study aims to analyze the in vivo activity of CM extract on insulin levels in streptozotocin-induced diabetic rats. By investigating the effects of CM extract on insulin levels, this research seeks to provide valuable insights into its potential as an adjuvant therapy for managing T2DM. The findings of this study may pave the way for the development of novel, plant-based therapeutic strategies that can complement existing treatments and improve the overall management of T2DM, ultimately reducing the burden of this chronic condition on individuals and healthcare systems.

2. Materials and Methods

2.1. Preparation of Samples and Research Subjects

Leilem leaves used in this research sample were taken from two areas, namely in Kayuuwi, West Kawangkoan District, Minahasa Regency, and Rumoong Atas, Tareran District, South Minahasa Regency; both areas are located in North Sulawesi Province, Indonesia. Samples were then brought to the Sam Ratulangi University Faculty of Medicine Test Laboratory, washed and dried. The research subjects used were male Wistar rats from the Makassar area, 2-3 months old, with a body weight of 150-200 grams, which met the criteria. The Health Research Ethics Committee permitted this research, Manado Health Polytechnic Ministry of Health No. KEPK.01/10/314/2023, October 02, 2023.

2.2. Simplicia and Extract Preparation

Leilem leaf samples have been picked, washed, and cleaned of foreign objects, then dried in a room not exposed to direct sunlight. After drying, it is pulverized using a blender until it becomes powder. The powder is then filtered to get fine powder and then weighed. Leilem leaves were extracted using the maceration method. A sample weighing 100 grams was placed in a glass jar and given a 96% ethanol solution until the powder sank, then closed and allowed to stand for 5 × 24 hours at room temperature and stirred. After that, the solution was filtered to obtain filtrate I. Then, filtrate I was again added with 96% ethanol solution, closed, and allowed to stand for 3 × 24 hours at room temperature and stirred. After that, it was filtered again for filtrate II. Filtrate I and II are mixed. The thick extract is obtained from evaporation results using an oven with a temperature of 50° C and allowed to stand at room temperature.

2.3. Preparation of Streptozotocin and CMC Solution

Streptozotocin was made at 40 mg/kg BW in rats weighing 200 g given 8 mg STZ per tail. Dissolving 0.4 g streptozotocin into citrate buffer with pH four as much as 50 ml. CMC is made by dissolving 0.5% CMC into 100 ml of distilled water, homogenized and heated, then cooled to room temperature.

2.4. Treatment of Research Subjects

The research subjects were 20 rats divided into four groups, each consisting of 5 rats. Group I served as the negative control group (CMC). Groups II and III were administered ethanol extract of CM leaf at doses of 250 mg/kgBW and 500 mg/kgBW, respectively. Group IV acted as the positive control group and received glibenclamide at a dose of 0.25 mg/kgBW. Before treatment and 48 hours after treatment, the animals were weighed, and their blood sugar levels were measured. Subsequently, each animal weighing 200 grams was given 8 mg/kg BW of streptozotocin by intraperitoneal injection to induce diabetes.

The treatment phase lasted for 14 days. During this period, Groups II and III received their respective doses of CM leaf ethanol extract (250 mg/kgBW and 500 mg/kgBW) orally using an oral syringe (sonde) and disposable tips. Group IV (positive control) received 0.05
mg of glibenclamide orally per rat weighing 200 g, using a sonde and disposable tips. Group I (negative control) received a CMC solution orally, using a sonde and disposable tips. All treatments were administered daily throughout the 14-day duration.

2.5. Collection and Examination of Serum Plasma of Research Subjects

Blood plasma serum from experimental animals was taken after the animals were terminated. After termination, blood was taken directly from the heart using a syringe and transferred to a red cap tube according to the label. Then, the tube was centrifuged at a speed of 2,000 - 3,000 x for 15 minutes. After centrifugation, the serum obtained was transferred to a separate tube. The serum samples were stored in the refrigerator/freezer at -20°C ± 5°C.

Insulin levels were examined through blood plasma serum from the centrifuged blood of the experimental animals that had been taken. Examination of elevated insulin levels was determined using the ELISA Kit (Rat/Mouse Insulin) procedure [16].

3. Results and Discussion

The CM leaf samples used in this study were fresh and clean. Five kg of CM leaf samples were washed using running water. The leaves were dried separately in a room without sunlight at 25°C. The CM extract was obtained from the maceration process. Extraction aims to obtain the desired content of chemical compounds present in the simplisia [17].

The experimental animals used in this study were male Wistar rats. The reason for using rats in this study is because rats are animals that have physiological conditions of the body that are similar to humans; not only physiological conditions, but the biological responses of rats are almost the same as humans, making them suitable for use in studies related to humans [18]. Before testing, Wistar rats were first acclimatized for ± 10 days. Acclimatization is necessary because the rats come from different locations and must adjust to the new climate. Different locations and climates can cause physiological and behavioral changes in an organism [19, 20]. The experimental animals used were induced/injected with diabetogenic agents in the form of streptozotocin to make the physiological conditions of Wistar rats become diabetic. This streptozotocin works by forming highly reactive free radicals that can certainly be alloxan, causing damage to cell membranes, proteins, and DNA and resulting in impaired insulin production by pancreatic beta cells [21]. Streptozotocin is used because it has a mechanism of action that causes selective damage and has lower side effects than alloxan [22].

In the negative test group, the animals were given Na-CMC solution. This 0.5% Na-CMC solution into 100 ml of distilled water, then homogenized and heated, then cooled to room temperature. Na-CMC has good stability in keeping drug particles evenly dispersed in the solution, has good viscosity, and is safe for test animals. While for the positive test group, the experimental animals were given glibenclamide. Glibenclamide is a drug that is useful for controlling high blood sugar levels in patients with T2DM. Glibenclamide stimulates the pancreas to increase the body's production and use of the hormone insulin. This hormone is responsible for getting blood sugar into the body's cells so that blood sugar levels can decrease [23].

The oral solution comprised ethanol extract of Ileilem leaves, glibenclamide, and Na-CMC 0.5%. It was administered once daily using a 1 mL syringe and disposable tip. Oral administration using a sonde is done because the method is relatively simple and easy to do on test animals [24]. The experimental animals were treated for 14 days, during which 14 days of treatment, the experimental animals were given water and food, weighed body weight, measured blood sugar levels, and given test solutions. During the 14 days of treatment, in each group of test samples, some animals died due to various factors [25].

3.1. Insulin Standard Measurement Results

The results of insulin standard measurements were obtained from absorbance values based on the concentration used from each solution; the standard concentrations used were 0.2 ng/mL, 0.5 ng/mL, one ng/mL, two ng/mL, five ng/mL and ten ng/mL.

In Table 1, the absorbance results of the insulin standard are obtained. These results show that the higher the concentration of insulin standard, the greater the absorbance value produced. This means that the higher the concentration, the more the amount of insulin captured in the standard increases. The standard concentration and absorbance results show a standard curve of insulin (Figure 1).

3.2. Analysis Result of Insulin Standard and Test Sample

The results of the standard insulin concentration and its absorbance value are described in a standard curve (Figure 1) so that a regression equation is obtained: y = 0.1141x + 0.1852 with a correlation coefficient of 0.8928. Based on the linear regression equation of insulin standard concentration and absorbance, the absorbance
of each sample group was taken based on the best sample absorbance, and the absorbance value was plotted into the regression equation obtained to obtain the sample insulin concentration.

3.3. Sample and Control Absorbance Measurement Results

The controls and samples used in this study were blood serum from Wistar rats induced by streptozotocin. There are four groups of experimental animals used, namely the negative control (CMC 0.5%) group (A), 250 mg/kgBW leilem leaf ethanol extract group (B), 500 mg/kgBW leilem leaf ethanol extract group (C) and positive control glibenclamide (10 mg/kgBW) group (D), (Table 2) and (Figure 2).

The research procedure used in the study used a procedure by the ELISA Kit (Rat/Mouse Insulin), where the procedure is designed to be very specific for the target molecule, in this case, to detect whether there is an increase in insulin levels in diabetic rats given ethanol extract. The kit is easy to use and comes with pre-coated plates and ready-to-use reagents. This assay usually gives relatively fast results compared to other methods [25, 26].

Leilem leaves come from the genus Clerodendrum L., and several studies have reported that this genus has antidiabetic potential [19]. Leilem leaves contain secondary metabolite chemical compounds such as alkaloids, saponins, flavonoids, steroids, and phenols; it is also reported that leilem is an antioxidant. This antioxidant ability of leilem protects pancreatic β cells from oxidative stress damage that can cause a decrease in insulin function [22].

The 250mg and 500mg ethanol extracts of leilem leaves used in this study became the solution tested to assess whether there was an increase in insulin in diabetic rats. Based on the ELISA Kit procedure used, at the final stage of the procedure, the insulin standard well forms a blue color with intensity proportional to the increase in insulin concentration. The higher the concentration, the bluer the color produced [25]. In Table 1, the absorbance results of the insulin standard are obtained. These results show that the higher the concentration of insulin standard, the greater the absorbance value produced. This means the higher the concentration of insulin standard, the greater the absorbance value produced.

In Table 1, the absorbance results of each sample insulin concentration produced, the greater the absorbance value produced. This means that the increase in insulin is significant in this group because this group is a comparison group (drug).

The data obtained from the negative control group (A) in Table 2 showed that the absorbance value was low. This means that insulin did not increase significantly in this group. From the 250mg/kg BW ethanol extract test group (B), it was found that the absorbance value was higher than the A group when the results were plotted in the linear equation formula to find the insulin concentration of the sample. This means that the increase in insulin was significant in this group. From the 500 mg/kg BW ethanol extract test group, it was found that the absorbance value was high but lower than the B group. The data obtained in the positive group (D) found that the absorbance value in this group was fairly good and higher when the results were plotted in the linear equation formula to find the sample insulin concentration. This means that the increase in insulin is significant in this group because this group is a comparison group (drug).
leaf extract contains an optimal concentration of active compounds that can stimulate insulin production in diabetic rats.

While this study provides valuable insights into the antidiabetic potential of CM leaf extract, it is important to acknowledge its limitations. The study employed only two variations of CM extract concentrations, and further research with a broader range of concentrations is necessary to establish the most effective dose for antidiabetic treatment. Additionally, more comprehensive studies, including in vitro, in vivo, and in silico approaches, are required to elucidate the underlying mechanisms of action and identify the specific compounds responsible for the antidiabetic effects of CM leaf extract.

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