THE EFFECT OF ETHANOLIC EXTRACT OF DAYAK ONION (Eleutherine palmifolia (L) MERR) TUBER ON BLOOD GLUCOSE AND INSULIN LEVEL OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RAT

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ABSTRACT

Objectives: The current investigation was conducted to examine the ability of Ethanolic extract from onion dayak (Eleutherine palmifolia) (L) (Merr) tuber to reduce blood glucose and increase insulin production in streptozotocin-induced diabetic wistar rat. Materials and Methods: Extraction was accomplished using maceration method with 96% ethanol. Antidiabetic activity was evaluated using observation of blood glucose levels utilize glucometer (Easy Touch). Analysis of insulin plasma concentration was determined utilize the Insulin Elisa Kit (Chem Cruz). Results: The administration of dayak onion extract as a form of therapy in diabetes, although it has not been able to increase insulin levels significantly, but has shown a tendency to increase insulin levels at higher doses of 500 mg/kg.

Conclusion: Ethanolic extract from dayak onion tuber exhibited activity of decrease blood glucose level and increase plasma insulin concentration in streptozotocin-induced diabetic wistar rat. Keywords: Eleutherine palmifolia, dayak onion tuber, diabetic, streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is one of the main problems of world health with a rapidly increasing incidence and the 6th largest cause of death in America. The World Health Organization predicts that DM patients will reach 5% of the world's population in 2025 (approximately 300 million people)1,2. In Indonesia, DM sufferers are approximately 8.4 million in 2004 and are projected to increase to 21.3 million in 2030. The number of people with DM in Indonesia ranks 4th largest in the world in 2016, India ranks first (31.7 million), followed by China (20.8 million) and America (17.7 million)3-4.

Type 2 DM is one type with the most patients among other forms of DM, even it reached 90% of the total incidence of DM in 2010. Worldwide, there are 135 million people with type 2 DM and are estimated to reach 300 million in 2025. The incidence of type 2 DM not only in adults but also in children, adolescents and young adults5-7. Insulin resistance is the main metabolic abnormality in most patients with type 2 DM, where both in animals and humans there is intracellular lipid accumulation in skeletal and liver muscles8,9. This accumulation of lipids causes GLUT4 inhibition, which then inhibits the entry of glucose into cells and inhibits glucose oxidation and glycogen synthesis10,11. Balanced diet and aerobic exercise is recommended for nonpharmacologic therapy for DM patients, while pharmacologic therapy is carried out using oral antidiabetic agents through stimulation of insulin secretion from pancreatic cells in patients who still have the ability to secrete insulin, and use exogenous insulin therapy which helps transport glucose from the blood into cells. If the nonpharmacologic therapy have not been able to reach the goal of therapy, pharmacologic therapy is recommended for people with DM, but the use of drugs can also have a negative effect for long time consumed12,13.

Dayak onion (Eleutherine palmifolia) (L) (Merr) is a typical Central Kalimantan, Indonesia, plant that has been...
used for generations by Dayaks as a medicine for various types of diseases including breast cancer, colon cancer, hypertension, DM, preventing strokes and lowering cholesterol. It is known that dayak onion contains secondary metabolites of alkaloids, flavonoids, glycosides and saponins which have hypoglycemic activity which is very useful for the treatment of DM. Flavonoids found in onion dayak are able to restore the function of pancreatic tissue by increasing insulin release through β cells, thereby reducing blood sugar levels and also can improve the sensitivity of peripheral cells to insulin. Other active compounds found in dayak onions are eleutherol, eleutherinosa A and eleuthoside B. Eleutherinosa A in dayak onion has the most role in overcoming DM, also reported to be able to inhibit alpha glucosidase enzyme (an enzyme that acts to break down starch and disaccharide into glucose). If alpha glucosidase activity is inhibited, then the availability of glucose outside the cell membrane is also hampered, so that glucose levels in the blood will decrease. The ethanol extract of dayak onion significantly reduced blood sugar levels in alloxan-induced DM rats.

However, less information is available about the effect of onion dayak ethanol extract on blood glucose levels, insulin levels and pancreatic histology. The study of antidiabetic effects by using experimental animals carried out by inducing animals to become diabetic, the chemical that become an inducer is streptozotocin (STZ). It is known that STZ affects pancreatic β cells and interferes with insulin production and results in increased blood glucose levels. Therefore, this study aims to prove the ability of onion dayak tuber extract can reduce blood glucose, increase insulin production and improve pancreatic β cells in STZ-induced diabetic wistar rats.

MATERIALS AND METHODS

Plant and chemicals materials

The current study was conducted in the Pharmacognosy Laboratory, Biology Laboratory and Pharmacology Laboratory, Faculty of Pharmacy, University of Sumatera Utara. And also Anatomy Pathology Laboratory, Faculty of Medicine, University of Sumatera Utara.

Onion dayak tuber (length of ± 5–7 cm and width of ± 1–2 cm) was collected (2 kg) from Laut Dendang village, Deli Serdang regency, Sumatera Utara province, Indonesia. Onion dayak was identified in Herbarium Medanense, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. (No. 4484/MEDA/2019). The apparatus used in this study were rotary evaporator, glassware, blenders, electric balance, simplicia dryers, porcelain bowls, desiccators, separating funnels, furnaces, animal scales, syringes, oral sonde, animal restrainers, rat cages, glucometers, glucostest strips, and Rat Insulin ELISA Kit (Chem Cruz).

Ethanol 96%, glibenclamide tablets; carboxymethyl cellulose (CMC) sodium 0.5%; glucose 50%; streptozotocin (Chem Cruz); concentrated sulfuric acid; reagent of Mayer and Dragendorff, chloriform, ammonium hydroxide; anhydrous acetic acid; FeCl3; sodium hydroxide; concentrated hydrochloric acid; methanol; formalin; aquadest.

Preparation of simplicia and ethanolic extract

The onion tuber were separated from the impurities and roots, then washed with running water, next drained and weighed as wet weight. Dayak onion tuber were cut into pieces then dried at 40°C and weighed as dry weight. Afterward, the material was pollinated using a blender. Simplicia was putted in a plastic container and tied, then etched and stored in a place protected from sunlight. The simplicia powder of dayak onion tuber (2kg) was macerated with 96% ethanol for 2 days and stirred several times. Next, the simplicia was filtered using filter paper. The simplicia was submerged for twice, until the limpid filtrate was obtained. Afterward, the filtrate was separated using rotary evaporator and the viscous extract was obtained. The viscous ethanolic extract of dayak onion tuber (EEDOT) were placed in a beaker glass and covered with aluminum foil, then stored in the freezer for damage prevention. The CMC (0.5%) were used as a solvents.

Experimental animals

Adult male rats (200–250 g) were used as an experimental animal and were placed and acclimatized in a well-ventilated animal transit room. (No.0499/KEPH-MIPA/2018)

Preparation of high lipid diet

The goat fat and the duck eggs were boiled. Afterward, the wheat flour (2 kg), granulated sugar (2 kg), standard pellets (2 kg), duck egg yolk (15 grains), bulk oil (1.5 kg) were mixed. The water of goat fat was added to the mixture and the dough batter was obtained. Next, added HFD before the rats induced by STZ.

Weight measurement of body weight and blood collection

Measurements of body weight were carried out in normal rats, diabetic rats (after STZ induction on days 0, 3, 6, 9, 12, 14), and rats after administration of extracts.

The rat blood was taken from tail-end. The tail was cleaned with alcohol (70%) and slashed with a razor blade. Afterward, the blood coming out was affixed to the glucometer strip that has been installed on the device. Next, the number on the screen was noted, and the rat tail-end was cleaned with alcohol (70%).

Blood glucose level measurement

The calibration strip was entered into the insertion strip. Next, the strip wrap was opened up to the sign line. Afterward, the strip was entered into the tool, then the strip wrap was removed. The serial number and the last blood sugar level were appeared on the screen, alternately. The blood was dropped to the strip tip. Next, the blood sugar levels were appeared on the screen after 30 seconds. The blood glucose levels was observed using glucometer (Easy Touch).

Antidiabetic test in streptozotocin-induced rat

The rats were fasted for 16 hours (water is still given). Afterward, the weight and blood glucose level of rats were measured (to determine the initial of body weight and blood glucose level). Next, injected with intraperitonial streptozotocin solution (dose of 60 mg/kg bw). The blood glucose level was measured (the value of ≥ 200 mg/dL was considered diabetes) on the third day. The experimental animals used in this experiment were divided into 6 groups.

- b. Group II (P2): Diabetic rats and given 0.5% wb the suspension of CMC 0.5% as a negative control.

- c. Group III (P3): Diabetic rats and given 0.5% wb the suspension of CMC 0.5% as a negative control.

- d. Group IV (P4): Diabetic rats and given 0.5% wb the suspension of CMC 0.5% as a negative control.

- e. Group V (P5): Diabetic rats and given 0.5% wb the suspension of CMC 0.5% as a negative control.

- f. Group VI (P6): Diabetic rats and given 0.5% wb the suspension of CMC 0.5% as a negative control.
c. Group III (P3): Diabetic rats and given the suspension of glibenclamide (dose of 10 mg/kg bw) as a positive control.
d. Group IV (P4): Diabetic rats and given EEDOT suspension of 125 mg/kg bw
e. Group V (P5): Diabetic rats and given EEDOT suspension of 250 mg/kg bw
f. Group VI (P6): Diabetic rats and given EEDOT suspension of 500 mg/kg bw

The solution was given orally for 15 consecutive days (group of 1-6). Afterward, the blood glucose levels were measured (at hour of 0, 1, 2, 3, 7) as a acute data and were premeasured (at day of 3, 6, 9, 12, 14) as chronic data. Subsequently, the experimental animals were dissected (on the 15th day) (Farid et al., 2014). The serum of experimental animals were taken for determining insulin levels (before dissection).

**Insulin Assay**

Analysis of insulin plasma concentration was determined utilize the Insulin Elisa Kit (Chem Cruz), and performed using the following procedure:

a) First reaction. The rat insulin in the sample was bound to anti-insulin antibodies coated on microplate wells and the unbound material was washed.
b) Second reaction. The POD conjugated with anti-insulin antibodies were bound to anti-insulin antibodies, anti-insulin antibodies of rat was mobilized to micro-washing wells and the excessive POD conjugated was washed.
c) Enzyme reaction. The bounded POD conjugate in the micro-plate well was detected using addition of 3,3',5,5'-tetrametibenidine (TMB) substrate solution. The absorbance was measured.
d) Evaluation of results. Insulin concentration was determined through interpolation using the standard curve produced by planning the absorbance value of the appropriate concentration of standard insulin.

**Statistical analysis**

Data were expressed as mean ± standard mean error (S.EM). The results were analyzed using one-way ANOVA then followed by Dunnet comparison test. The results were statistically significant at p <0.05.

**RESULTS AND DISCUSSION**

**Weight measurement of body weight**

Significant weight gain of rats was occurred from 1st to 4th week. The average of body weight was showed the following data:

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (gram)</th>
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<tbody>
<tr>
<td></td>
<td>Begin</td>
</tr>
<tr>
<td>P1</td>
<td>158.85</td>
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<tr>
<td>P2</td>
<td>166.12</td>
</tr>
<tr>
<td>P3</td>
<td>160.85</td>
</tr>
<tr>
<td>P4</td>
<td>164.92</td>
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<tr>
<td>P5</td>
<td>161.25</td>
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<tr>
<td>P6</td>
<td>167.83</td>
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</tbody>
</table>

The previous study has reported that rats fed with the High Fat Diet (HFD) method lead to condition of insulin resistance that correlates with significant weight gain.

**Blood glucose level measurement**

The blood glucose levels were shown an increase in exceeded the significant normal range. According to the previous report, group of both positive control and treatment were experienced of hyperglycemia with blood glucose levels above 250 mg/dL. The average of blood glucose level for whole group (at day of 3rd, 6th, 9th, 15th) was presented in Table 2. The group of P2, P4, P5 and P6 were induced with streptozotocin (STZ) that had a higher increase in blood glucose level compared to P3 group (negative control). As shown in Table 1, the blood glucose level (at 15th day) was decreased in group of P1 (positive control) and P6 (EEDOT of 500 mg/kg bw), that lower than value of 100 i.e. (83.1 ± 8.84) and (93.66 ± 9.72) which is near to normal blood glucose level (87.0 ± 2.82) in group P1. Meanwhile, the blood glucose level was decreased in P4 (EEDOT of 125 mg/kg bw) and P5 (EEDOT of 250 mg/kg bw) which is in the range value of 100 i.e. (125.8 ± 26.3) and (101.1 ± 15.3). Next, the difference between each treatment group was assessed utilize the Post Hoc Test using SPSS 16.

Based on the statistics, blood glucose levels between groups were significantly different, where a significant decrease was occurred in the group of P4 (EEDOT of 125mg/kg bw), P5 (EEDOT of 250 mg/kg bw) and P6 (EEDOT of 500 mg/kg bw). A study by Dewi and colleague (2016) demonstrated that the group received EEDOT dose of 500 mg/kg bw has decreased in blood glucose level.

Decreasing blood glucose level in the positive control group occurs due to the body's own healing mechanism physiologically through the repair of pancreatic β cells and new cell division (mitosis) which takes place gradually. While the decrease in blood glucose level in the treated group given EEDOT can prevent oxidation of pancreatic β cells so that reduced the damage. Bioactive compounds contained in EEDOT based on phytochemical screening was performed as described previously, include alkaloids, flavonoids, glycosides, and saponins that has hypoglycemic activity or decrease blood glucose levels that useful for the treatment of diabetes mellitus. Flavonoid in dayak onion can recover the function of pancreatic tissue through increasing insulin release by β cells, which lowers blood sugar levels and improve the sensitivity of peripheral cells to insulin.

The blood glucose level was increased (at 3rd day) and decreased (at 15th day) in the group of P2 (positive control), P4 (EEDOT of 125 mg/kg bw), P5 (EEDOT of 250 mg/kg bw) and P6 (EEDOT of 500 mg/kg bw). This increase may due to food factors and increases after eating that usually at the lowest level in the morning before breakfast. High glucose levels in the blood (hyperglycemia) without good control will cause one of the endocrine diseases, i.e. diabetes mellitus. In addition, diabetes mellitus can also be influenced by physiological stress and environmental stress, where stress will increase adrenaline then inhibits insulin activity that lead the blocked of lowering blood glucose level.
The administration of dayak onion extract as a form of therapy in diabetes, although it has not been able to increase insulin levels significantly, but has shown a tendency to increase insulin levels at higher doses of 500 mg/bw.

CONCLUSION

Ethanolic extract from dayak onion tuber exhibited activity of decrease blood glucose level and increase plasma insulin concentration in streptozotocin-induced diabetic wistar rat. The most effective dayak onion extract in reducing blood glucose levels and increasing insulin plasma concentration significantly at a dose of 500 mg/kg bw. This finding might emphasize the potency of dayak onion extract as an antidiabetic. Nevertheless, a farther inquiry is still required to define the detailed mechanism of the antidiabetic activity of dayak onion extract.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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