Potential Combination of Tinospora crispa, Andrographis paniculata, Cinamomum burmanii, Syzygium polyanthum and Momordica charantia Extracts Against Intake of Glucose in Muscle Rats-Induced Diabetes Mellitus

Rachmat Hidayat1*, Patricia Wulandari2

1Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
2Cattleya Mental Health Center, Palembang, Indonesia

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Corresponding author:
Rachmat Hidayat
E-mail address:
dr.rachmat.hidayat@gmail.com

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1. Introduction

Diabetes mellitus is a chronic condition that disturbs the body's blood sugar regulation. This disorder is characterized by a decrease in the ability of body cells to intake glucose into cells. Due to the failure of cells in glucose intake, glucose buildup occurs in the extracellular, namely in the plasma. These medicinal plants contain quite varied secondary metabolites, where these plants are rich in flavonoids, alkaloids, terpenes and glycosides. The content of these secondary metabolite compounds is believed to be rich in antioxidant effects so that it has the effect of being able to suppress various oxidative stress conditions that cause damage to various organs due to blood sugar dysregulation. The effect of Tinospora crispa, Andrographis paniculata, Cinamomum burmanii, Syzygium polyanthum and Momordica charantia extracts on blood sugar levels and GLUT4 protein expression in muscle tissue shows the potential of the test extract's ability to improve blood glucose intake to cells so as to maintain blood sugar regulation.

Tinospora crispa, Andrographis paniculata, Cinamomum burmanii, Syzygium polyanthum and Momordica charantia are one of the most common plants in Indonesia. This plant is often found in various
regions in Indonesia, where these plants are often in the form of shrubs or wild plants that grow in yards or plantations. These medicinal plants contain quite varied secondary metabolites, where these plants are rich in flavonoids, alkaloids, terpenes and glycosides. The content of these secondary metabolite compounds is believed to be rich in antioxidant effects so that it has the effect of being able to suppress various oxidative stress conditions that cause damage to various organs due to blood sugar dysregulation.

This study aims to assess the effects of the extracts of Tinospora crispa, Andrographis paniculata, Cinamomum burmanii, Syzygium polyanthum and Momordica charantia on blood sugar levels and GLUT4 protein expression in muscle tissue which shows the potential of the test extract's ability to improve blood glucose intake to cells so as to maintain sugar regulation blood.

2. Methods

Animal model

A total of 30 white rats (Rattus norvegicus) Wistar strain obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 - 250 grams. All experimental animals were kept in cages under controlled conditions of 12 hours of light dark cycle, temperature 22 ± 1°C and humidity 40-60% and given ad libitum food. The research treatments and procedures have received approval from the medical research ethics committee of the Faculty of Medicine, Sriwijaya University (No. 243 / kptfkunsri-rsmh / 2020).

Herbal combination extraction preparation

Herbal Simplicia was obtained from the Tawangmangu Herbal Research Center, Karanganyar, Indonesia. The process of extracting sambiloto is carried out by maceration in which 500 grams of simplicia are macerated with 96% ethanol for 72 hours. Next, do the separation between the pulp and the macerate. The masure was then evaporated with a rotary evaporator (Shimadzu) in order to obtain a thick extract, herbal combination extract (EKH).

Animal model of diabetes mellitus

After 1 week of adaptation, the mice were randomly divided into the following six groups, each containing 5 animals: Normal control group, diabetes group (negative control), diabetes + metformin group (Met; 45 mg / kg), Diabetes + ES group (50 mg / kg), diabetes + ES group (100 mg / kg) and diabetes + ES group (200 mg / kg). Induction of diabetes was done by injecting alloxan at a dose of 110 mg / kgBW intraperitoneally, then white mice were given 10% glucose to drink. The positive control group was treated with metformin (Dexa Medica, Indonesia) for 14 days. In the treatment group the extract of sambiloto was carried out for 14 days. The mice were anesthetized by injecting 10% Chloral Hydrate (3.5 ml / kg) intraperitoneally. Rats were sacrificed by injecting 10% chlorine hydrate, then blood serum was taken through the orbital vein and the femoral muscle was taken from the thigh of the white rat. The serum was then centrifuged at 10.000 rpm for 10 minutes, at 25°C and the supernatant was stored at -20°C for analysis of blood sugar levels using the spectrophotometer method (Biorad). Meanwhile, the muscle tissue was evacuated, some of which were homogenized and centrifuged to obtain a supernatant and put it in a later RNA solution (Sigma Aldrich) and stored at -20°C, for ELISA examination of GLUT4 protein.

Enzyme-linked immunosorbent assays (ELISA) GLUT 4

GLUT4 levels in joint synovial fluid were examined with Rat ELISA GLUT4 (Cloud Clone), based on the protocol contained in the manufacturer's protocols. Briefly, 50 μl of standard diluent or serum samples were added to the well coated with anti-GLUT4 and incubated at 37°C for 30 minutes. After the plates were washed, 100 μl of the biotinylated antibody solution was added and incubated for 30 minutes at 37°C. After washing three times, 50 ul of avidin-peroxidase
complex solution was added and incubated for 15 minutes at 37°C. After washing, 50 μl of tetramethylbenzidine color solution was added and incubated in the dark for 15 minutes at 37°C. Finally, 50 ul stop solution was added to stop the reaction, and the optical density (OD) was measured using an ELISA reader (Biorad), wavelength of 450 nm.

**Phytochemical test**

The extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids / triterpenoids. The extract was separated using TLC as a stationary phase in the form of silica gel GF254 and the mobile phase in the form of n-hexane: chloroform: ethyl acetate (2: 5: 5).

**Statistical analysis**

All data were presented as mean ± standard deviation and all statistical analyzes were performed with the SPSS 25 (IBM) program. One way ANOVA followed by post hoc analysis was carried out to assess the difference in mean expression levels of each protein. P <0.05 was determined as an indication that there was a significant difference in mean levels.

### 3. Results and Discussion

Table 1 shows the potential of the extract on blood sugar levels of White Rats. Alloxan-induced white rats showed a very significant increase in blood sugar levels, where the use of the drug metformin was able to reduce blood sugar levels significantly even though they had not reached the target blood glucose target, which was less than 200 mg / dL. The treatment with the combination extract was able to significantly reduce blood sugar levels, where at the EKH 100 and 200 mg / KgBW doses it was able to reduce blood sugar levels to reach the target below 200 mg / KgBW.

Table 2 shows the levels of GLUT4 in muscle tissue, where the GLUT4 protein is an important transporter in the regulation of glucose intake into cells. Increased expression of GLUT4 in a tissue indicates an increase in the ability of cells to intake glucose. In white rats induced with diabetes mellitus, there was a decrease in GLUT4 levels in muscle tissue. The administration of metformin drugs or sambiloto extracts has shown the ability to increase GLUT4 protein levels.

Table 3 shows the secondary metabolite content of the combined extract. The combination extract is rich in flavonoids. The dominant flavonoids in bay leaf extract are believed to be responsible for the effect of blood glucose regulation.

The content of flavonoids is the main secondary metabolite compound which is believed to play a role in blood glucose regulation. Flavonoids increase the expression of GLUT 4 protein in muscle tissue. Increased expression of GLUT 4 causes an increase in glucose intake into cells. Increased glucose intake into cells, causes a decrease in the buildup of glucose outside the cells and interstitial tissue, which leads to a decrease in blood glucose levels.

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>Blood Glucose (mg/dL) ± SD</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Con</td>
<td>28.26 ± 3.41</td>
<td>0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetes</td>
<td>496.23 ± 15.43</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetes + Met</td>
<td>97.41 ± 7.21</td>
<td>0.00</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetes + EKH 50</td>
<td>379.12 ± 21.43</td>
<td>0.00</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetes + EKH 100</td>
<td>279.11 ± 18.65</td>
<td>0.00</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetes + EKH 200</td>
<td>163.83 ± 10.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* VS Diabetes + Met; ANOVA, pos hoc Bonferroni; p<0.05
### Table 2. Level of GLUT 4 in Muscle

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>GLUT4 (pg/mL) ± SD</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Con</td>
<td>228.26 ± 3.41</td>
<td>0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetes</td>
<td>56.23 ± 15.43</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetes + Met</td>
<td>147.41 ± 7.21</td>
<td>0.00</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetes + EKH 50</td>
<td>86.12 ± 21.43</td>
<td>0.00</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetes + EKH 100</td>
<td>108.11 ± 18.65</td>
<td>0.00</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetes + EKH 200</td>
<td>145.83 ± 10.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* VS Diabetes + Met; ANOVA, pos hoc Bonferroni; p<0.05

### Table 3. Phytochemical Test

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Saponins</th>
<th>Alkaloids</th>
<th>Triterpenoids</th>
<th>Steroids</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>EKH</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++++</td>
</tr>
</tbody>
</table>

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### 4. Conclusion

The herbal combination extract has an effect in lowering blood sugar levels in Diabetes Mellitus White Rats by increasing glucose intake to cells and tissues.

### 5. References


