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The Effects of Sambiloto Leaf Extract *(Andrographis peniculata)* on Blood Sugar Regulation: an In Vivo Study

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ABSTRACT

Sambiloto (*Andrographis peniculata*) is one of the most common plants in Indonesia. Sambiloto contains quite varied secondary metabolites, where this plant is rich in flavonoids, alkaloids, terpenes and glycosides. This study aims to assess the effect of Sambiloto (*Andrographis peniculata*) leaf extract on blood sugar levels and the expression of GLUT4 protein in muscle tissue which indicates the potential of the test extract's ability to improve blood glucose intake to cells so that it can maintain blood sugar regulation. The process of extracting sambiloto is carried out by maceration in which 500 grams of simplicia are macerated with 96% ethanol for 72 hours. 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). The treatment with sambiloto extract was able to reduce blood sugar levels significantly, were at the ES 100 and 200 mg/kg BW doses it was able to reduce blood sugar levels to reach the target below 200 mg/kg BW. The dominant flavonoids in Sambiloto leaf extract are believed to be responsible for the effect of blood glucose regulation. In conclusion, Sambiloto extract affects lowering blood sugar levels in diabetes mellitus white rats by increasing glucose intake to cells and tissues.

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 the body's cells to intake glucose into cells. Due to the failure of cells in glucose intake, glucose buildup occurs in the extracellular, namely in plasma. The buildup of glucose in the plasma causes an increase in blood glucose levels in the plasma so that it will interfere with blood flow, which leads to blood viscosity and decreased blood flow to various cells and tissues. Impaired blood flow to various cells and tissues leads to a decrease in the supply of oxygen and nutrients to cells and tissues resulting in damage and cell death which leads to decreased tissue performance.

Sambiloto (*Andrographis peniculata*) is 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. Sambiloto contains quite varied secondary metabolites, where this plant is 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 effect of Sambiloto (*Andrographis peniculata*) leaf extract on blood sugar

levels and the expression of GLUT4 protein in muscle tissue which indicates the potential of the test extract's ability to improve blood glucose intake to cells so that it can maintain blood sugar regulation.

2. Research 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, Universitas Sriwijaya (No. 187/kptfkunsri-rsmh/2020).

Sambiloto extraction preparation

Simplisia sambiloto 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 maserate was then evaporated with a rotary evaporator (Shimadzu) in order to obtain a thick extract, bitter extract of Sambiloto (ES).

Animal model diabetes melitus

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/kg BW intraperitoneally; then the white rats 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 anaesthetized by injecting 10% Chloral Hydrate (3.5 ml/kg) intraperitoneally. The rats were sacrificed by intraperitoneal injection of 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 25oC, and the supernatant was stored at -20oC 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 -20oC, for ELISA examination of GLUT4 protein.

Enzyme-linked immunosorbent assays (ELISA) GLUT 4

GLUT4 levels in joint synovial fluid were checked 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 three washing, 50 ul of avidin-peroxidase complex solution was added and incubated for 15 minutes at 37˚C. After washing, 50 µl of tetramethylbenzidine colour 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), the wavelength of 450 nm.

Phytochemical test

The Sambiloto extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids/triterpenoids. The ethyl acetate fraction 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 Sambiloto leaf 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 of less than 200 mg/dL. The treatment with sambiloto extract was able to reduce blood sugar levels significantly, were at the ES 100 and 200 mg/kg BW doses it was able to reduce blood sugar levels to reach the target below 200 mg/kg BW.

Table 2 shows the levels of GLUT4 in muscle tissue, where the GLUT4 protein is an essential 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 bitter extract. Sambiloto extract is rich in flavonoids. The dominant flavonoids in Sambiloto leaf extract are believed to be responsible for the effect of blood glucose regulation. The content of flavonoids in the leading secondary metabolite compound believed to play a role in blood glucose regulation. Flavonoids increase the expression of the 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 1. Level of Blood Glucose in Serum

No.	Group	Blood Glucose (mg/dL) ± SD	P Value*
1.	Con	28.26 ± 3.41	0.00
2.	Diebetes	496.23 ± 15.43	-
3.	Diabetes + Met	97.41 ± 7.21	0.00
4.	Diabetes + ES 50	386.12 ± 21.43	0.00
5.	Diabetes + ES 100	298.11 ± 18.65	0.00
6.	Diabetes + ES 200	155.83 ± 10.12	0.00

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

Table 2. Level of GLUT 4 in Muscle

No.	Group	GLUT4 (pg/mL) ± SD	P Value*
1.	Con	228.26 ± 3.41	0.00
2.	Diabetes	56.23 ± 15.43	-
3.	Diabetes + Met	147.41 ± 7.21	0.00
4.	Diabetes + ES 50	76.12 ± 21.43	0.00

5.	Diabetes + ES 100	98.11 ± 18.65	0.00
6.	Diabetes + ES 200	135.83 ± 10.12	0.00

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

Table 3. Phytochemical test

Ingredients	Saponin	Alkaloid	Triterpenoid	Steroid	Flavonoid
ES	+	-	+++	++	+

4. CONCLUSION

Sambiloto extract affects lowering blood sugar levels in diabetes mellitus white rats by increasing glucose intake to cells and tissues.

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