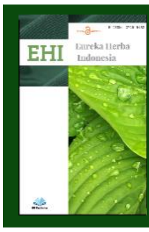

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Journal Homepage: https://eurekabiomedical.com/index.php/EHI

Effect of Bay Leaf Extract (*Syzygium polyanthum*) on Blood Sugar Regulation via GLUT4 Protein Regulation in Rat Muscle Tissue Induced Aloxan

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ARTICLE INFO

Keywords:
Diabetes mellitus
Terpenes
Alloxan
Syzygium
Blood glucose

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/ehi.v1i1.4

A B S T R A C T

Diabetes mellitus is a chronic condition that disturbs the body's blood sugar regulation. Bay leaves contain entirely various secondary metabolites, and this plant is rich in flavonoids, alkaloids, terpenes, and glycosides. This study aims to assess the effect of bay leaf extract (*Syzygium polyanthum*) on blood sugar levels and the expression of GLUT4 protein in muscle tissue. A total of 30 white rats (Rattus norvegicus) Wistar strains were obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 – and 250 grams. Bay leaf Simplicia was obtained from the Tawangmangu Herbal Research Center, Karanganyar, Indonesia. 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 + BLE (75 mg/kg), diabetes + BLE group (150 mg / kg) and diabetes + BLE group (300 mg/kg). 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 bay leaf extract was able to reduce blood sugar levels significantly. The administration of metformin drugs or bay leaf extract showed the ability to increase the level of GLUT4 protein. In conclusion, bay leaf extract affects reducing blood sugar levels in diabetes mellitus white rats by increasing glucose intake in 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 decreases 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.[1-4]

Bay leaf (Syzygium polyanthum) 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. This plant is very often used as a spice in cooking and various cooking mixtures. Bay leaves contain entirely various secondary metabolites, and 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.[5-9]

This study aims to assess the effect of bay leaf extract (Syzygium polyanthum) 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 in cells to maintain blood sugar regulation.

2. Methods

Animal model

A total of 30 white rats (Rattus norvegicus) Wistar strains were obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 – and 250 grams. All experimental animals were kept in cages under controlled conditions of 12 hours of the 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. 187/kptfkunsri-rsmh/ 2020).

Bay leaf extraction preparation

Bay leaf Simplicia was obtained from the Tawangmangu Herbal Research Center, Karanganyar, Indonesia. The process of extracting bay leaf 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 macerate was then evaporated with a rotary evaporator (Shimadzu) to obtain a thick extract, bay leaf extract (BLE).

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 + BLE (75 mg/kg), diabetes + BLE group (150 mg / kg) and diabetes + BLE group (300 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 bay leaf extract was carried out for 14 days. The mice were anesthetized 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 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 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 the 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), a wavelength of 450 nm.

Phytochemical test

The bay leaf extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids/triterpenoids. The bay leaf 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 bay 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 bay leaf extract was able to reduce blood sugar levels significantly, were at the EDS 150 and 300 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 bay leaf extract showed the ability to increase the level of GLUT4 protein.

Table 3 shows the secondary metabolite content of bay leaf extract. Bay leaf 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 in the leading secondary metabolite compound is 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 in 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.[10-14]

Table 1. Level of blood glucose in serum

No.	Group	Blood Glucose (mg/dL) ± SD	P-Value*
1.	Control	97.36 ± 8.41	0.00
2.	Diabetes	389.23 ± 15.43	-
3.	Diabetes + Met	209.11 ± 10.21	0.00
4.	Diabetes + BLE 75	216.12 ± 21.43	0.00
5.	Diabetes + BLE 150	185.11 ± 17.65	0.00
6.	Diabetes + BLE 300	143.83 ± 12.21	0.00

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

Table 2. Level of GLUT 4 in muscle

No.	Group	GLUT4 (pg/mL) ± SD	P-Value*
1.	Control	218.26 ± 3.41	0.00
2.	Diabetes	58.23 ± 15.43	-
3.	Diabetes + Met	157.41 ± 7.21	0.00
4.	Diabetes + BLE 75	79.12 ± 21.43	0.00
5.	Diabetes + BLE 150	99.11 ± 18.65	0.00
6.	Diabetes + BLE 300	125.83 ± 10.12	0.00

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

Table 3. Phytochemical test

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

4. Conclusion

Bay leaf extract affects reducing blood sugar levels in diabetes mellitus white rats by increasing glucose intake in cells and tissues.

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