TEST OF BAY LEAF EXTRACT (*Syzygium polyanthum*) AS AN ANTIDIABETIC USING MOUSE TEST ANIMALS (*Mus musculus*)

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A B S T R A C T

Diabetes mellitus is a cause of hyperglycemia. Diabetes mellitus is the most common cause of hyperglycemia, while other factors can also cause it. Diabetes mellitus causes sugar to accumulate in the blood, preventing it from entering cells. Bay leaves are woody plants that are often harvested for their leaves. Bay leaves have traditionally been used as a spice. There are many ways to use bay leaves as traditional medicine. Bay leaves have extraordinary therapeutic abilities and are commonly used in the treatment of hypertension and diabetes. Mice are commonly used as experimental animals in scientific research because of their short life cycle, large number of offspring per birth, high variation in traits, ease of handling, and the ability to detect anatomical and physiological features. The purpose of this study was to determine the decrease in antidiabetic in mice (Mus musculus) by administering bay leaf extract (Syzygium polyanthum) induced by glucose. The type of research used an experimental method (Quantitative Research) to test bay leaf extract (Syzygium polyanthum) as an antidiabetic drug in mice (Mus musculus). The results of this study found that giving bay leaf extract at a dose of 6 mg/20 g body weight, 20 mg/20 g body weight, and 33 mg/20 gs body weight can reduce blood glucose levels in mice for 7 days. This study found that bay leaf extract (Syzygium polyanthum) significantly reduced blood sugar levels in mice, with a value of (10.779<2.352.).

INTRODUCTION

Diabetes mellitus is a metabolic disease caused by an unhealthy lifestyle. According to data *from the International Diabetes Federation* (IDF), one person dies from diabetes every 7 seconds. Furthermore, diabetes affects one in twelve people, and one in two people with diabetes are unaware of their condition (IDF, 2015). Today, the increasing number of people with diabetes can be caused by a variety of risk factors in addition to a poor lifestyle. According to data obtained from the *American Diabetes Association* (ADA), risk factors that cannot be changed are family history of people with diabetes mellitus, age over 45 years, ethnicity, history of giving birth to babies with a birth weight of more than 4000 g or diabetes eruptions, and low birth weight (<2.5 kg) (Soelistijo, 2019).

Diabetes mellitus first broke out in Indonesia in 2013 and is estimated to have affected 12 million people, with 3.7 million untreated. According to Riskesdas (2013), this potential will grow to reach 21.3 million people by 2030. Diabetes can cause a variety of consequences, including diabetic cardiomyopathy, visual impairment (blindness), kidney failure, and lower limb amputation. In this condition, blood glucose levels increase resulting in an increase in oxidative stress mechanisms in cells, including heart muscle cells, resulting in the death of heart cells due to the release of toxic substances, disrupting the function of the heart muscle, and increasing diastolic final pressure which can ultimately lead to heart failure (Juwita & Febrina, 2018).

The disorders mentioned above can be fatal, including death. In addition to death, diabetes can cause disability due to complications. Prompt treatment of diabetes can keep blood glucose levels normal and prevent the risk of serious consequences (Lestari et al., 2021). However, nowadays most people are afraid of the side effects of the diabetes medications they take, so they turn to herbal remedies to manage their condition. According to Lestari et al. (2021), Asia has the largest distribution of antidiabetic herbal plants in the world, reaching 56%. Bay leaf (*Syzygium polyanthum*) is one of the herbal plants that can be used as an alternative to diabetes treatment. According to the book Wijayakusuma (1994), bay leaves have therapeutic properties on all parts of the plant, including

the bark, roots, fruits, and leaves. However, the therapeutic properties of its leaves are much greater than the therapeutic properties of other components (Ramadhan et al., 2023).

Up to 56% of herbal plants are believed to be able to lower blood glucose levels. Bay leaf (Syzgium polyathum) is a plant used to treat diabetes mellitus. Bay leaves are generally used as an additional cooking spice (Widiyono, 2020).

METHODOLOGY

Type of Research Using an experimental method (Quantitative Research) to test bay leaf extract (*Syzygium polyanthum*) as an antidiabetic drug in mice (*Mus musculus*).

Time and Place

The study was conducted from June 29 to August 10, 2024. The Pharmacy Laboratory of Baubau Polytechnic and the Pharmacy Laboratory of Mandala Waluya University are the locations of this research.

Tools and Materials

This study uses tools such as stirring rods, blenders, bunsen, funnels, beakers, and measuring cups. Glucometers, scissors, flannel, animal cages, tripods, measuring flasks, lumps and pestles, digital balances, droppers, cutter knives, sondes, spatula, glucometer strips, syringes hermometers and animal scales. This study used ingredients such as aquadest, bay leaf (*Syzygium polyanthum*), ethanol 95%, mouse (*Mus musculus*), sodium carboxyl methyl cellulose (Na-CMC), glibenclamide 5 mg and glucose.

Research Procedure

Sample Preparation

The leaves of the green bay (*Syzygium polyanthum*) are cut into small pieces and washed thoroughly. Then, without direct sunlight, dry it with air for 14 days or until it is completely dry. Next, add bay leaves. Dried bay leaves (*Syzygium polyanthum*) are then ground in a blender until smooth. Bay leaf powder (Syzygium polyanthum) is sifted.

Preparation of Bay Leaf Extract (*Syzygium polyanthum*)

Then 200 g of bay leaf powder is dissolved into 95% ethanol as much as 2.5 L. Maceration is carried out in maceration containers at room temperature for three days, with periodic stirring, before being precipitated and poured for two days. The solution is then filtered using filter paper, and maceration is collected. The maceration is then evaporated using *a rotary vacuum evaporator* to produce a viscous extract.

Glucose Solution Manufacturing

Weigh 100 mg of glucose**.** Dissolve with 25 mL of 0.5 % Na-CMC into a beaker**.** Stir until homogeneous.

Preparation of Na-CMC Solution 0.5 %

Weighed Na-CMC 0.5% as much as 0.25 g. Put it in a beaker**.** Dissolve with aquadest as much as 50 mL.

Preparation of bay leaf extract solution

Stir until homogeneous. Weighed 100 mg, 300 mg and 500 mg of bay leaf thick extract**.** Put in a beaker**.** It is dissolved using 15 mL of 0.5% Na-CMC solution**.**

Preparation of Glibenchamide Solution

Stir until homogeneous. Glycenclamide was weighed as much as 0.013 mg**.** It is dissolved using a 0.5% Na-CMC solution of 20 mL**.** Stir until homogeneous.

Induction of Diabetes in Experimental Animals

All mice were fasted on the first day. Next, check the blood sugar levels of mice using a glucometer. Digest 1 mL of glucose solution for 4 days. A glucose solution is given orally to mice to induce diabetes. After receiving a glucose solution for 24 hours, the blood sugar levels of the mice were measured on days 1, 4 and 7.

Administration of the extract to mice

In this study, three mice were given treatment for each concentration of test animals. Bay leaf extract was given according to the treatment dose, which was 1 mL in each mouse. Group 1 (Negative control group) was given a 0.5% Na CMC solution**.** Group 2 (Positive control group) was given glibenchamide 0.00065 mg/20 g BB. Group 3 (Test group) was given bay leaf extract with a dose of 6 mg/20 g BB. Group 4 (Test group) was given bay leaf extract at a dose of 20 mg/20 g BB. Group 5 (Test group) was given bay leaf extract at a dose of 33 mg/20 g BB. Oral swabs with dispo are used once every 2 days for 7 days for each oral concentration.

Measurement of Blood Sugar Levels in Mice

Before treatment, the animals were trained to adapt for approximately one week. Injecting a glucose solution into the mouths of mice produces diabetes. Glucose is given once as much as 1 mL. Then check blood sugar levels through blood sampling by slightly trimming the tip of the tail. The gut and tail are sterilized using alcohol. A glucometer is used to measure blood pressure. In less than 10 seconds, the glucometer strip is filled with blood and the results are displayed on the screen. The value of blood sugar concentration in mg/dl is displayed on the screen.

Data Analysis

The data will be analyzed with the Anova test. This study uses the anova two-step test data analysis technique in Microsoft Excel.

RESULTS AND DISCUSSION

Research Results

Blood Sugar Level Data of Mice in the Negative Control Group

The results of the negative control group of mice who were given treatment for 7 days. On the first day to the fourth day, the mice were given a 1 mL glucose solution once a day orally, then on the fifth to seventh day for positive control mice were given Na CMC at a dose of 0.5 % mL/20 g BB once a day orally.

Table 4.1 Blood sugar levels of mice in the control group were negative during 7 days of treatment.

group	Mouse blood sugar (mg/dl)		
negative control (Na-CMC)	**Day 1**	**Day 4**	**Day 7**
1	**80**	**137**	**95**
2	**105**	**155**	**112**
3	**125**	**156**	**90**

Source : Primary Data, 2024

Blood Sugar Level Data of Positive Control Group Mice

The results of the positive control group of mice were given treatment for 7 days. On the first to fourth day, the mice were given a 1 mL solution of aloxan once a day intraperitoenally, then on the fifth to seventh day for negative control mice were given Na-CMC 0.5 % mL once a day orally and positive control mice were given Glibenclamide at a dose of 0.00065 mg/20 g BB three times a day orally.

Table 4.2 Blood sugar levels of mice in the positive control group during 7 days of treatment.

group	Mouse blood sugar (mg/dl)		
positive control (Glibenchamide)	**Day 1**	**Day 4**	**Day 7**
1	**75**	**125**	**90**
2	**82**	**139**	**112**

3	**115**	**155**	**134**

Source : Primary Data, 2024

Blood Sugar Level Data of Test Group 1 Mice

The results of the test group 1 mice were given treatment for 7 days. On the first to fourth day, the mice were given a 1 mL glucose solution once a day orally, then on the fifth to seventh day, the mice were given bay leaf extract at a dose of 6 mg/20 g BB (100 mg/g BB) once a day orally.

Table 4.3 Blood sugar levels of test group I mice during 7 days of treatment.

Group	**Mouse blood sugar (mg/dl)**		
Bay leaf extract Dosage 2 mg	**Day 1**	**Day 4**	**Day 7**
1	**139**	**179**	**148**
2	**110**	**155**	**112**
3	**158**	**178**	**136**

Source : Primary Data, 2024

Data on Blood Sugar Levels of Mice in Test Group II.

The results of the study of test group II mice were given treatment for 7 days. On the first to fourth day, the mice were given a 1 mL glucose solution once a day orally, then on the fifth to seventh day, the mice were given bay leaf extract at a dose of 20 mg/20 g BB (300 mg/g BB) once orally.

Table 4.4 Blood Sugar Levels of Test Group II Mice During 7 Days of Treatment.

Group	**Mouse blood sugar (mg/dl)**		
Bay leaf extract Dosage 6 mg	**Day 1**	**Day 4**	**Day 7**
1	**100**	**188**	**130**
2	**125**	**147**	**103**
3	**109**	**154**	**138**

Source : Primary Data, 2024

Blood Sugar Level Data of Test Group III Mice

The results of the study of test group III mice were given treatment for 7 days. On the first to fourth day, the mice were given a 1 mL glucose solution once a day orally, then on the fifth to seventh day, the mice were given bay leaf extract at a dose of 33 mg/20 g BB (500 mg/g BB) once a day orally.

Table 4.5 Blood Sugar Levels of Test Group III mice during 7 days of treatment.

group	**Mouse blood sugar (mg/dl)**		
Bay leaf extract Dosage 10 mg	**Day 1**	**Day 4**	**Day 7**
1	**226**	**264**	**174**
2	**142**	**153**	**121**
3	**120**	**158**	**137**

Source : Primary Data, 2024

Table 4.6 Statistical Test of ANOVA *one-way* Blood Sugar Levels in Mice

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*Anonymous*
Between Groups	42538.72	15	2835.915	10.779	0.000	2.352
Within Groups	4209.5	16	263.094			
Total	46748.22	31				

Source : Primary Data, 2024

Information:

Ss = Number of squares due to the source

Df = Degree of Freedom from the Source
Ms = The average number of squares due to the source
F = Statistics (F calculate)
p-value = p value
F crit = Statistics (F table)

Discussion

Bay leaf extract (*Syzygium polyanthum*) was tested as an antidiabetic drug in rats (Mus musculus). The mice were given glucose to lower their blood sugar levels. Bay leaf extract is given to healthy male rats (Mus musculus) aged 2-3 months with a body weight ranging from 20-30 g. The mice, in addition to being readily available, were sensitive enough to measure the blood glucose levels of these test animals, since their metabolic and digestive systems were comparable to those of humans.

Samples for this study were taken from 08:00 to 10:00 WITA. This is done in the morning because in the morning the plant stomata open this is related to transpiration and photosynthesis, two examples of plant metabolism. During the process of photosynthesis, stomata serve as the outlet of cell fluids and participate in the diffusion of CO2. In this study, green bay leaves (*Syzygium polyanthum*) were cut into small pieces, washed, and dried for 14 days or until dried without exposure to direct sunlight. Dried bay leaves (*Syzygium polyanthum*) are ground with a blender until smooth. Bay leaf powder (*Syzygium polyanthum*) is sifted.

In this study, 15 mice (*Mus musculus)* were used as test animals. They were divided into five groups: the positive control group, the negative control group, the test group I, the test group II, and the test group III. The positive control group was given 1 mL of glucose solution orally once a day for 4 days, followed by the positive control group was given 0.00065 mg of glibenchamide orally 3 times a day for 3 days. The negative control group was given 1 mL of glucose solution orally once a day for 4 days, followed by oral administration of 0.5 ml of Na-CMC once a day for 3 days. In test group I, 1 mL of glucose solution orally once a day for 4 days, then 6 mg/20 g (100 mg/g BB) of bay leaf extract was given orally once a day for 3 days. In test group II, 1 mL of glucose solution orally once a day for 4 days, then 20 mg/20 g (300 mg/g BB) of bay leaf flower extract was given orally once a day for 3 days. In the III test group, 1 mL of glucose solution orally once a day for 4 days, then 33 mg/20 g (500 mg/g BB) of bay leaf extract was given orally once a day for 3 days.

Administration of bay leaf (*Syzygium polyanthum*) with doses of 6 mg, 20 mg and 33 mg was used to lower blood sugar levels in mice, glibenchamide was used to lower blood sugar levels and Na-CMC as a controller.

Blood sugar level measurement in mice was carried out 3 times, namely on days 1, 4 and 7. The normal blood sugar level of mice is 73 mg/dl-96.6 mg/dl, and mice are declared diabetic when their blood sugar level has reached ≥180 mg. Table 4.11 is the result of the ANOVA statistical test study one, the control group is different from the test group I, II, and III. In addition, it is known that celery leaf extract can lower blood glucose levels in mice. From the blood sugar level data of the negative and positive control groups, test 1, test 2 and test 3 for the first day all groups were fasted, then a blood sugar level test was carried out using a glucometer and then a glucose solution was given for 4 days after being given, then on the 4th day the blood sugar level of mice was checked again so that it was found out whether the blood sugar level increased or not. After being given a glucose solution and blood sugar levels have been checked, the next stage that is carried out is the administration of drugs and extracts in each group for 3 days, then on the 7th day the blood sugar levels of mice in all groups are measured again, the blood sugar levels of all groups decreased, so the results obtained have a significant value.

In this study, the extract made is an extract from bay leaves that have gone through various processes and produce a thick extract of bay leaves. The doses given to mice were test group I with a dose of 6 mg/20 g of mouse body weight, test group II with a dose of 20 mg/20 g of mouse body weight, and test group III with a dose of 33 mg/20 g of mouse body weight.

CONCLUSION

The results showed that bay leaf extract (*Syzygium polyanthum*) could reduce blood sugar levels in mice by obtaining significant values (10,779<2,352). This study has limitations such as the

environmental conditions where mice are kept such as temperature, humidity, and animal stress can affect the results of the study, so it can be a source of data variations that are not well controlled

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