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Research Article

Antihyperglycemic Effectiveness of Ethanolic Extract of Takokak Fruit (Solanum Torvum Swartz) on Male White Rats (Rattus Norvegicus) Induced by Glucose

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Abstract: Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose levels resulting from impaired insulin secretion, insulin resistance, or a combination of both. This condition requires long-term treatment and has the potential to lead to serious complications if not properly managed. The search for alternative herbal-based treatments has become a major focus of various pharmacological studies. One such plant with potential is the takokak fruit (Solanum torvum Swartz), which has traditionally been used to treat various health conditions such as hypertension, microbial infections, and reproductive system disorders, as well as possessing antipyretic and antidiabetic properties. This study aimed to evaluate the antihyperglycemic efficacy of the takokak fruit ethanol extract in glucose-induced male white rats (Rattus norvegicus). The extract was obtained through a maceration method using 96% ethanol as a solvent. Five test groups were prepared, each consisting of three rats: a negative control group (Na-CMC), a positive control group (metformin), and three treatment groups with takokak extract concentrations of 5%, 10%, and 20%. Glucose induction was administered orally, and after 30 minutes, the extract was administered to the treatment group, and blood glucose levels were measured for two hours. The results showed that the takokak fruit extract was able to reduce blood sugar levels by 27% in the 5% group, 25% in the 10% group, and 33% in the 20% group, respectively. However, the ANOVA statistical test yielded a significance value of 0.48, indicating that the difference in blood glucose reduction between the groups was not statistically significant. Although not yet significant, these results indicate that the ethanol extract of takokak fruit has potential as an antihyperglycemic agent, which requires further research on a larger scale and duration to obtain more conclusive results.

Keywords: Antihyperglycemic, Blood Sugar Level, Glucose induction, Male white rats, Takokak.

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1. Introduction

Diabetes mellitus is a disease that affects energy production in human cells. Abnormalities in insulin secretion or activity cause this condition, resulting in hyperglycemia (Kharrobi, & Darwish, 2015) . An increase in fasting glucose levels of more than 129 mg/dL indicates hyperglycemia. Hyperglycemia is characterized by problems processing carbohydrates, fats, and proteins in the body. In people with diabetes, hyperglycemia occurs because insulin cannot work effectively, causing glucose to be unable to enter muscle cells, fat tissue, and the liver (Dolanggeng, 2017) .

Hyperglycemia can be reduced with pharmacological therapy, as well as with natural medications. Natural-based medications are reported to be safer and have a lower risk of side effects compared to synthetic drugs (Handayani & Mahanani, 2019) . Takokak fruit is one natural ingredient that can be used to lower blood glucose levels (Anwar et al ., 2017) .

Takokak fruit has traditionally been used by the community as an antidiabetic by cooking, boiling, and using it as a side dish (Yuli & Yuni, 2021) . Takokak fruit extracted using ethanol contains secondary metabolites, including tannins, alkaloids, saponins, steroids, glycosides, and flavonoids (Helilusiatiningsih & Soenyoto, 2020) . Flavonoids are

found in most plants in Indonesia, one of which is the takokak fruit plant, which has uses as an antidiabetic (Alfaridz & Amalia, 2018) . Flavonoids show effectiveness as antidiabetic agents due to their antioxidant function, where these compounds can renew pancreatic β cells while supporting the stimulation of insulin secretion (Kumalasari et al , 2020) . Diabetes mellitus conditions in test animals can be induced with chemicals such as glucose. Test animals that are overloaded with glucose without damaging their pancreas can experience a more rapid increase in blood sugar levels, which can be reduced with substances that have antihyperglycemic effects (Ivanka, 2021) . Male white mice were used in this study because, males' bodies show better biological stability than females, in addition, hormonal factors and pregnancy do not affect their bodies, so the research results are more effective and reliable (Lahamendu et al , 2019) .

This study was conducted with the aim of assessing the ability of ethanol extract of takokak fruit to reduce blood sugar levels in male white rats (Rattus norvegicus) that had been induced by glucose.

2. Research Methods

Tools and materials

The equipment used in this study includes a beaker, pestle and mortar, stirring rod, funnel, measuring cup, filter paper, parchment paper, watch glass, horn spoon, thermometer, glass container, sonde for mice, syringe, stopwatch, glucometer, hot plate, aluminum foil , blood sugar measuring strips, mouse cage, as well as scales for mice and analytical scales. In this process, distilled water and ethanol extract derived from takokak fruit (Solanum torvum Swartz), 96% ethanol, glucose, Sodium Carboxymethyl Cellulose (Na-CMC), and metformin were used.

Research Procedures

Sample Preparation

A 4kg sample of nearly ripe takokak fruit, dark green and of good quality, was taken from a plantation in Bengkol Village, Mapanget District, Manado City, North Sulawesi Province.

Sample Processing

The collected takokak fruit is first wet sorted, then washed thoroughly using running water. The takokak fruit is split into two parts, to speed up the drying process. To dry the takokak fruit, the fruit is placed in a container made of woven bamboo with small pores and covered with black plastic, to avoid direct exposure to sunlight. Once completely dry, the samples are sorted to remove dirt that adhered during the drying process, and to select good-quality simplicia. The simplicia is placed in a closed glass container to continue the maceration process (Usman & Fikifandry, 2019) .

Making Thick Takokak Fruit Extract

Takokak fruit powder was weighed as much as 250 grams, then the maceration method was used in this extraction, by soaking the simplicia in 1875 ml of 96% ethanol for 72 hours with occasional stirring. The maceration solution was then filtered using filter paper. After that, the filtrate was heated using a water bath at a temperature of 50°C until a thick extract was obtained and ready for use (Yulianto & Annik, 2018) .

Making Takoka Fruit Extract Suspension

Suspensions from takokak fruit extract were prepared at concentrations of 5% (equivalent to 1 gram), 10% (2 grams), and 20% (4 grams). The extract was weighed according to the desired dosage, then mixed into 20 ml of 1% Na-CMC solution, and stirred using a stirring rod until evenly mixed (Pane, 2021) .

Preparation of 1% Na-CMC Suspension

Na-CMC weighing 1 gram was added little by little into 50 ml of hot distilled water at a temperature of <100°C, while stirring continuously using a stirring rod until evenly mixed, then the volume was completed to 100 ml with distilled water (Dolanggeng, 2017) .

Preparation of Metformin Suspension

Metformin is administered to humans at a dose of 500 mg. The conversion factor used to convert the dose to rats is 0.018, meaning the metformin dose for rats is 9 mg (the result of multiplying 500 mg by 0.018). 0.204 grams of ground metformin was weighed and mixed evenly with 20 ml of 1% Na-CMC solution (Mongi et al , 2019) .

Making Glucose Suspension

50 grams of glucose was put into a 100 ml beaker glass , then suspended with 100 ml of 1% Na-CMC until homogeneous (Dolanggeng, 2017).

Grouping of Test Animals

This study used male white mice weighing between 104 and 171 grams. The mice were divided into five groups, with three mice in each group. The first group was given 1 g of Na-CMC per kg of body weight, the second group received a dose of metformin 0.204 g/kgBW. The third group was given takokak fruit extract with a concentration of 5% and a dose of 1 g/kgBW, the fourth group received a 10% extract of 2 g/kgBW, and the fifth group received a 20% extract with a dose of 4 g/kgBW.

Antihyperglycemic Testing

This study involved 15 white male mice divided into five groups. Before treatment, all mice fasted for 12 hours, but were still given water to drink. Their body weight was measured and marked, then fasting blood sugar levels were measured. Glucose injections were given orally at a dose of 50 g/kgBW to each mouse, and blood sugar levels were remeasured after 30 minutes. After that, each group was given oral treatment: the first group received Na-CMC at 1 g/kgBW, the second group metformin at a dose of 9 mg/kgBW, and the test group of takokak fruit extract was divided into three extract dose groups: 5% at a dose of 1 g/kgBW, 10% at a dose of 2 g/kgBW, and 20% at a dose of 4 g/kgBW. Blood sugar levels were checked again at 30, 60, and 120 minutes. Blood was taken from the mouse's tail vein and analyzed using a GlucoDr device. (Togubu et al , 2013) .

3. Results And Discussion

Takokak Fruit Extraction Results

The results of takokak fruit extraction can be seen in table 1.

Table 1. Results of 96% Ethanol Extraction of Takokak Fruit (Solanum torvum Swartz)

Sampel	Jenis pelarut	Volume pelarut (ml)	Berat sampel (gram)	Berat ekstrak (gram)	Rendemen (%)
Buah takokak (*Solanum torvum* Swartz)	Etanol 96%	1.875	250	12,03	4,8

Table 1 shows that 12.03 grams of thick extract was obtained with a yield of 4.8%. According to the Indonesian Herbal Pharmacopoeia, a good yield is <7.2%, while the resulting yield was 4.8%. The yield obtained was low. Research by Wijaya et al. (2022) states that prolonged extraction will result in a high yield. This is due to the prolonged contact between the material and the solvent, which allows more compounds to diffuse out of the cells.

Antihyperglycemic Effectiveness Test Results

The antihyperglycemic test was conducted using the oral glucose tolerance method on white male mice. Based on research conducted by Jeanne (2021) , the oral glucose tolerance test aims to measure the ability of mice to manage glucose, which has an important role as energy. In this study, 15 white male mice were used as experimental objects and were grouped into five groups, where the first group served as a positive control and received a Na-CMC suspension at a dose of 1 g/kg body weight, the second group as a negative control received a metformin suspension at a dose of 0.204 g/kgBW, while the test group was given an extract suspension with a concentration of 5% (dose 1 g/kgBW), 10% (dose 2 g/kgBW), and 20% (dose 4 g/kgBW). Observations were carried out for 30, 60, and 120 minutes . All treatment groups were given the test solution orally. The antihyperglycemic test procedure refers to the research of Togubu et al (2013) . Before the test began, the mice first underwent a fast for approximately 12 hours. Fasting is done with the aim of reducing blood sugar levels by eliminating interfering factors such as food consumption that can affect blood sugar measurement results (Kumalasari et al , 2020) .

Hyperglycemia was induced by glucose inducing all mice. Research by Galuh (2021) stated that glucose was used as an induction agent to avoid damage to the pancreas of the test animals, and the glucose levels obtained were temporary, so they would be reduced with substances with antihyperglycemic effects. Mice were considered

hyperglycemic when their blood glucose levels were >127 mg/dL (Pane, 2021). Average blood sugar levels in male white mice can be found in Table 2.

Table 2. Average Results of Blood Sugar Level Measurements in Male White Mice

Kelompok perlakuan	Kadar gula darah (mg/dL				
	Gula darah puasa	Setelah induksi	Menit ke-30	Menit ke-60	Menit ke- 120
Na-CMC (-)	95	154,66	129,66	125,33	90,66
Metformin (+)	78,66	193,66	153	118,3	96,66
Ekstrak konsentrasi 5%	94	165,33	132,33	127	103,66
Ekstrak konsentrasi 10%	66	182,33	147,33	145,33	114
Ekstrak konsentrasi 20%	83,66	183,33	146,33	114,33	108

Based on table 2, all treatment groups were in the normal fasting blood glucose range of 66-95 mg/dL, so the mice used were in normal condition. In line with the research of Soriton et al , (2014) which stated that fasting blood glucose levels are usually in the range of 50-109 mg/dL. After 30 minutes of glucose administration, all treatment groups showed an increase in glucose levels in the body, especially the group receiving metformin as a positive control, which obtained the highest increase in blood sugar levels of 193.66 mg/dL. The test group of 20% concentration takokak fruit extract obtained the second highest increase in blood sugar levels, after the positive control which was 183.33 mg/dL, and followed by 10% concentration takokak fruit extract of 182.33 mg/dL, after that 165.33 mg/dL in 5% concentration takokak fruit extract, and In the negative control group given Na-CMC, blood sugar levels The recorded blood sugar level was 154.66 mg/dL. This is in line with research by Soriton et al (2014) which states that mice are said to be hyperglycemic when their blood sugar levels are >127 mg/dL. Test animals experiencing hyperglycemia have characteristics such as increased blood sugar levels and polyuria , which can be seen through the condition of the cage which is always moist (Indrawati et al , 2015) . The % change in blood sugar levels is shown in Table 3.

Table 3. % Decrease in Blood Sugar Levels of Male White Mice

Kelompok perlakuan	%Penurunan Kadar Gula Darah (%)			Rata-Rata (%)
	T_{30}	T_{60}	T_{120}	
Na-CMC (-)	16	19	41	25
Metformin (+)	22	40	50	37
Ekstrak Konsentrasi 5%	20	23	37	27
Ekstrak Konsentrasi 10%	19	20	37	25
Ekstrak konsentrasi 20%	20	37	41	33

From the results of table 3, the calculation of the percentage of reduction in blood sugar levels is done by subtracting the blood sugar value after induction with the sugar level at a certain minute, then dividing it by the blood sugar level after induction and the result is multiplied by 100% (Pane, 2021). From table 4.3, it can be seen that during the two hours of observation, the decrease in blood sugar levels in all test groups showed an increase at the 30th, 60th, and 120th minutes. In the group receiving Na-CMC, the decrease in blood sugar levels reached 16% at the 30th minute, experienced a change at the 60th minute of 19% and at the 120th minute there was an increase of 41%. In the 30th minute after administration of metformin suspension, there was the highest increase in blood sugar levels, namely 22%, then in the 60th minute it increased to 40%, and continued to increase to 50% in the 120th minute. The most striking increase in blood glucose was seen in the 60th and 120th minutes in the group given metformin compared to the other test groups.

The test group given 5% concentration of takokak fruit extract at the 30th minute experienced an increase in blood sugar levels as large as the test group with 20% concentration extract, namely 20%, then at the 60th minute it became 23%, and a change of 37% at 120 minutes. Takokak fruit extract with a concentration of 10% at the 30th minute was 19%, after the 60th minute it increased to 20%, and further increased to 37% at 120 minutes which was the same as the 5% concentration extract group. At the 30th minute, the extract with a concentration of 20% resulted in a decrease in blood sugar levels of 20%. Furthermore, at the 60th minute, blood sugar levels experienced the largest spike, namely 37%, which exceeded the other treatment groups. At the 120th minute, the same spike as the negative control group was recorded at 41%. Figure 2 presents an illustration of the average percentage decrease in blood sugar levels.

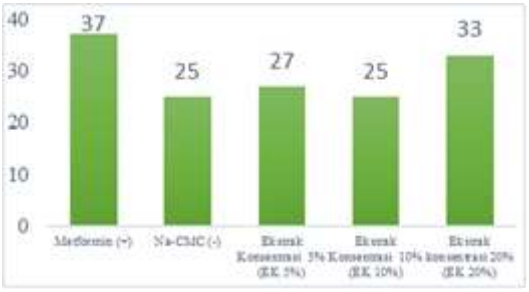

Figure 2. Average Percentage Reduction in Blood Sugar Levels in Mice

Based on Figure 2, the average percentage reduction in blood sugar in the negative control using Na-CMC reached 25%. The use of Na-CMC as a negative control is considered appropriate. According to a study by Indrawati et al. (2015) , experimental animals do not contain the cellulase enzyme in their digestive tracts, so Na-CMC does not affect blood sugar levels. However, this study found that the negative control reduced blood sugar levels by 25%, which is almost the same as the group given takokak extract at a concentration of 5%.

The positive control group showed the highest reduction in blood sugar, namely 37%. Metformin as a comparison is considered appropriate. Based on research conducted by Fadah & Nugraningsih (2020) , it states that Metformin is the most frequently used antihyperglycemic drug currently, and does not cause hypoglycemia, which is a condition when blood sugar levels fall below normal limits. The mechanism of action of metformin includes reducing glucose production in the liver and increasing insulin response in muscle and fat tissue. In addition, metformin also increases glucose utilization in the small intestine, so that blood sugar levels decrease due to reduced glucose absorption in the digestive tract after eating (Ludong et al , 2019) . In accordance with research conducted by Mongi et al (2019), it states that metformin is used as a comparison and has the highest reduction in blood sugar levels.

The results of this study indicate that administering takokak fruit extract with different concentrations has an impact on reducing blood sugar levels in mice that have been induced by glucose. The group receiving the extract with a concentration of 20% experienced the most significant average reduction in blood glucose levels, namely 33%, however, at a concentration of 5% it provided a higher reduction of 27%, compared to the extract with a concentration of 10%, namely 25%. Based on research conducted by Rasyad et al (2017) using strawberries (Fragaria ananassa) stated that increasing the dose of administration correlated with an increased ability to reduce blood glucose levels. The takokak fruit extract with the greatest antihyperglycemic effect and almost the same as metformin, was found in the 20% concentration extract group. The contributing factor is the high content of substances at that concentration, which allows for a more significant reduction in blood sugar levels.

The decrease in blood glucose in male white mice was caused by the presence of flavonoid compounds. This is in line with Kusuma's (2012) research , which stated that the results of phytochemical screening of takokak fruit contained flavonoid compounds. Flavonoids work by increasing insulin sensitivity in muscle and adipose tissue to lower blood sugar levels. Flavonoids can interfere with glucose absorption in the small intestine by inhibiting the breakdown of carbohydrates into glucose (Wulandari, 2016) . These results are consistent with a study by Zahra et al. (2021) which stated that flavonoid compounds showed that the flavonoid content in cherry leaves (Muntingia calabura) has the ability to lower blood sugar levels in mice. Therefore, the effect of flavonoids is almost the same as metformin, which works by increasing the effectiveness of insulin in muscle and fat tissue, as well as reducing glucose absorption in the small intestine.

In this study, statistical analysis was performed using SPSS with the ANOVA parametric test used to compare the average between two or more groups. Before applying the ANOVA test, certain steps are required, normality and homogeneity tests are carried out as mandatory requirements in parametric tests. Data must meet these two conditions, namely normal and homogeneous, to proceed with the ANOVA test. The Shapiro-Wilk normality test was applied because the sample size was less than 50, which shows that the data is normally distributed, indicated by a p-value above 0.05. Furthermore, the Levene test was carried out to check the homogeneity of the data to ensure that the samples come

from the same population, with significant results > 0.05 indicating homogeneity. Based on the results of the ANOVA test which showed a value of 0.48, which met the criteria for significance > 0.05, it was concluded that the ethanol extract of takokak fruit (Solanum torvum Swartz) was proven to have a blood sugar-lowering effect on white male rats.

4.Conclusion

Based on the research conducted, it was concluded that takokak fruit extract acts as an antihyperglycemic agent in glucose-induced mice. The 20% extract concentration had the strongest effect in lowering blood sugar levels compared to the other extract groups, with a significance value of 0.48.

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