The test effect of glucose tolerance of green apple (Mallus sylvestris Mill.) juice on male white mice

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ABSTRACT

Diabetes Mellitus or diabetes is a disease characterized by an increase in blood sugar levels beyond normal limits due to disruption of carbohydrate metabolism due to a deficiency of the insulin hormone caused by impaired pancreatic function. Glucose tolerance is disturbed when a person's blood sugar level on the glucose tolerance test is above normal but not high enough. Green Apple (Mallus sylvestris Mill.) Glucose Tolerance Test. Against White Male Mice orally by measuring mice's blood sugar levels every 30 minutes for 3 hours at a dose of 1.12 g; 2.24 g; and 4.48 g. The study's results using the oral glucose tolerance test method showed that the glucose tolerance effect of green apple juice at a dose of 1.12 g averaged blood sugar levels from 160.33 mg/dl to 89.33 mg/dl, at a dose of 2.24 g. – average blood sugar level from 204.67 mg/dl to 122.33 mg/dl and a dose of 4.48 g average blood sugar level from 329.67 mg/dl to 175.00 mg/dl. At a dose of 4.48 g, it can provide a faster effect on lowering the glucose levels of the test animals from statistical testing utilizing the T-test obtained t count ≤ t table means that there is no significant difference between the test substance group and the normal group.

Keywords:
Glucose Tolerance
Green Apple (Mallus sylvestris Mill.)
Mice

INTRODUCTION

Diabetes mellitus (DM) or diabetes is a disease characterized by an increase in blood sugar levels due to a disturbance in the body's metabolic system, in which the pancreas cannot produce the insulin hormone according to the body's needs (Nugroho, 2015). This metabolic disorder is caused by a lack of production or resistance of the body's cells to insulin. Low body insulin conditions result in excess sugar in the blood, called hyperglycemia(Galicia-Garcia et al., 2020). Common symptoms experienced by diabetics include fatigue, thirst (polydipsia), frequent hunger (polyphagia), frequent urination (polyuria), and decreased body weight (Fatimah, 2015). Glucose tolerance is disturbed when a person's blood sugar level on the glucose tolerance test is above normal but not high enough. Normal blood sugar levels are 80-90 mg/dl during fasting and will increase to 120-140 mg/dl 2 hours after eating (Care, 2013;DepkesRI, 2005).
The drug commonly used for diabetics is metformin. This drug has side effects of nausea, vomiting, and digestive disorders (Panamuan et al., 2021). To avoid side effects, people turn to traditional medicine. One alternative to lower blood sugar levels is to consume green apples (Mallus sylvethris Mill). Green apples contain chemicals such as flavonoids (quercetin), saponins, and vitamins, which lower blood glucose levels. In a previous study, a test for impaired blood glucose tolerance was carried out in white male rats due to dexamethasone's side effects by comparing red and green apple juice at a dose of 7.602g/200gBW (Khotimah, 2016).

RESEARCH METHOD

Test Kit
1 cc oral syringe, analytical scales, triple balance scales, a set of cages for mice, measuring cups, beaker glass, vials, test tubes, stir bars, DR glucose, funnels, dropping pipettes, DR glucose test strips, vaporizers, tissues, markers, measuring cup, scissors, flannel cloth, cloth, a place for drinking mice, a place for eating mice.

Ingredient
1. Green apple
2. Aqua dest
3. Glucose 50%, flannel

Test animals
The test animals used were 12 male white mice weighing 20 - 40 grams

Samples And Sample Techniques
1. Sampling
The sample used was a green apple (Mallus sylvethris Mill.) obtained from Bukittinggi, West Sumatra province. The sampling technique of this research is simple random sampling.

2. Sample Processing
Green apples are washed and air-dried. Cut green apples into small pieces, weigh as much as 300 grams, blend, strain, and put in a beaker glass.

3. Preparation of Test Substance Dosage
The planned doses are 8 g/200gBW, 16 g/200gBW, and 32g/200Gbb The conversion rate from mice to mice = 0.14. So that the first dose was 1.12 g/20gBW, the second dose was 2.24 g/20gBW, and the third dose was 4.48 g/20Gbb.

4. 50% Glucose Production
Weigh 50 grams of glucose, put it into a 100 ml measuring flask, dissolve it with distilled water until 100 ml, and shake until homogeneous.

5. Preparation of Test Animals
In this study, the test animals used were male white mice weighing 20-40 grams using the oral glucose tolerance test method. Before the treatment, the animals were acclimated for approximately seven days to adapt the mice to their new environment (Trisna et al., 2022); the test animals fasted for 16 hours with the aim of faster absorption of the preparations. Test animals were induced to increase fasting glucose levels with 50% glucose 3g/kgBW orally, except for the normal group (Khotimah, 2016).

Blood glucose levels were measured every 30 minutes for 3 hours using a blood glucose meter (Nesco multi-check) and test strips. The tip of the mouse's tail was cut and then pressed from the
base to the tip of the tail so that blood could be drawn; then, the blood was placed on a test strip, and the results of the determination could be obtained within 10 seconds.

6. **Testing for Decreased Blood Sugar**

Test animals were fasted for 16-18 hours but were still given water to drink (Trisna et al., 2022). The weight test animals were weighed, and blood sugar levels were measured in all groups. All groups except the normal group were given 50% glucose 3g/kgBW (0.12ml/20gBW). 30 minutes after giving glucose, the blood sugar level of the test animals was checked again. Oral administration was carried out as follows:

Group I (normal control) was given 0.5 ml/20 gBB of aqua dest. Group II test solution dose I 1.12g/20gBB. Group III test solution dose II 2.24g/20gBB. Group IV test solution dose III 4.48g/20gBB. 30 minutes after administration of the test preparation, check the blood sugar of the test animals at 30, 60, 90, 120, 150, and 180 minutes for 3 hours.

7. **Data Analysis Techniques**

Test the normality of the data using the Lilifort test. Variance homogeneity test using Levene’s test. One-way variance test. The T-test was carried out by a Ho rejection test on one-way anava (Mulyati et al., 2022; Fika, 2020).

**RESULTS AND DISCUSSIONS**

**Results**

From this study, the following results were obtained. Blood glucose levels in the test animal group that was given the green apple juice test solution were as follows:

1. The test solution at a dose I of 1.12 g/20gBW averaged blood sugar levels after administration of 50% glucose from 160.33 mg/dl to 89.33 mg/dl.
2. The test solution at a dose II of 2.24 g/20gBW averaged blood sugar levels after administration of 50% glucose from 204.67 mg/dl to 122.33 mg/dl.
3. The test solution dose III of 4.48 g/20gBB average blood sugar levels after administration of 50% glucose from 329.67 mg/dl to 175.00 mg/dl.

**Discussion**

Green apples contain flavonoids (quercetin) (Setiawan et al., 2018), which have activity in lowering blood sugar levels by regenerating pancreatic beta cells, increasing insulin secretion, and increasing cell sensitivity to insulin. Another mechanism of flavonoids that shows a hypoglycemic effect is reducing glucose absorption and regulating the activity of the expression of enzymes involved in carbohydrate metabolism (Kurnia, 2020). One of the flavonoid compounds, quercetin, affects the lowering of blood sugar levels. Meanwhile, polyphenols can help lower blood sugar levels because they function as antioxidants which are thought to be able to protect pancreatic beta cells (Fitriani et al., 2014).

In a previous study, a glucose tolerance test was carried out with the side effects of dexamethasone on green apple juice and red apple juice at a dose of 7.602 g / 200 g BW. This study showed that green apple juice reduced blood sugar levels in test animals from 288.71 mg/dl to 108.71 mg/dl compared to red apple juice (Khotimah, 2016).

This study used green apples with a dose of 1.12 g/20gBW, 2.24 g/20g, and 4.48 g/20gBW. The test solution at a dose I of 1.12 g/20gBW, the test solution at a dose II of 2.24 g/20gBW, and the test solution at a dose III of 4.48 g/20gBW showed a decrease in blood glucose levels in the test animals.
Table 1. The average fasting blood glucose level of animals for 3 hours

<table>
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</tbody>
</table>

Information:
Group name = 1, 2, 3, 4
Time (per 30 minutes) = 30 minutes to 180 minutes

Based on the Table 1, the blood sugar levels of the mice increased after being given 50% glucose and decreased after being given treatment with green apple juice. After administration of green apple juice, blood sugar levels had an average test dose I of 89.33 mg/dl, test dose II 122.33 mg/dl, and test dose III 175.00 mg/dl. This shows that giving green apple juice lowers blood sugar levels after being induced by 50% glucose.

In increasing blood glucose levels, glucagon stimulates glycogenolysis (the breakdown of glycogen into glucose), increases the transport of amino acids from the muscles, and increases gluconeogenesis (the formation of glucose from non-carbohydrates). In fat metabolism, glucagon increases lipolysis. In decreasing blood glucose levels, insulin acts as an anabolic hormone by increasing the diffusion of glucose through cell membranes in tissues. The anabolic effects of insulin hormone include inhibiting glycogenolysis, gluconeogenesis, and ketogenesis in the liver, increasing gluconeogenesis in bones and decreasing lipolysis in adipose tissue (Badoiu et al., 2021; Dewi et al., 2017).

The liver converts protein into glucose and replaces used glycogen (Trisna et al., 2017). When blood glucose levels increase, glucose is converted into fat through the process of lipogenesis. Still, when blood glucose levels fall, the liver helps maintain blood glucose concentrations by breaking down glycogen (glycogenolysis) and forming new glucose (gluconeogenesis) from amino acids and glycerol. And lactic acid (Han et al., 2016). The liver plays an important role in maintaining blood glucose level. If blood glucose levels increase (hyperglycemia), glucose is converted and stored as glucagon and fat. Through glycogenesis, lipogenesis, glycogenolysis, and gluconeogenesis, the liver helps maintain normal blood glucose levels and prevents hyperglycemia and hypoglycemia (Dewi et al., 2017).

The test solution at a dose of 1.12 g/20 gBW average blood sugar levels after administration of 50% glucose from 160.33 mg/dl to 89.33 mg/dl. The test solution at a dose of 2.24 g/20gBW
averaged blood sugar levels after administration of 50% glucose from 204.67 mg/dl to 122.33 mg/dl. The test solution dose of 4.48 g/20gBB average blood sugar levels after administration of 50% glucose from 329.67 mg/dl to 175.00 mg/dl.

The data normality test used statistical tests, namely Lilliford, and the data obtained was received $H_0$ (data normally distributed). Levene's test was used to test the homogeneity of variance, and the data obtained was rejected $H_0$. Meanwhile, in the Anava test, it was obtained $F_{count} \leq F_{table}$ at a significant level of $\alpha = 0.01$ and continued with the T-test that there was no significant difference between the 1st and 3rd test substance groups. So it can be concluded that green apple juice (Mallus sylvestris Mill.) has a glucose tolerance effect because the test substance given 50% glucose can lower blood glucose in test animals given green apple juice (Mallus sylvestris Mill.) with doses 1 and 3.

CONCLUSION

Based on the study's results, it was shown that giving Green Apple (Mallus sylvestris Mill.) juice could reduce blood sugar levels quickly at a dose of 4.48 grams/20gBB. Blood sugar levels can be brought back to normal by administering Green Apple Juice (Mallus sylvestris Mill.) at doses of 1.12 g/20gBB and 2.24 g/20gBB.

References


Han, H. S., Kang, G., Kim, J. S., Choi, B. H., & Koo, S. H. (2016). Regulation of glucose metabolism from a liver-centric perspective. Experimental and Molecular Medicine, 48(3), 1-10. https://doi.org/10.1038/emm.2015.122


