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THE EFFECTIVENESS OF BHEE FRUIT (MELASTOMA Sp) WATER EXTRACT ON BLOOD GLUCOSE LEVELS

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Abstract
Diabetes is a non-communicable disease with a prevalence that is increasing globally and nationally. Diabetes is characterized by blood glucose levels exceeding normal limits (>200 mg/dl). Blood glucose levels must be controlled to prevent serious complications in the human body. One method of controlling blood glucose levels is to use herbal medicines derived from plants. Exploration of herbal plants as antidiabetic agents remains limited. The Bhee fruit (Melastoma sp) is a widely distributed plant in Aceh, particularly in West Aceh, yet it has not been extensively utilized. The Bhee fruit holds potential as a herbal medicine; however, research confirming its antidiabetic properties remains scarce, necessitating further studies on its potential. This study aimed to determine the effectiveness of Bhee fruit water extract in lowering blood glucose levels in STZ-induced diabetic mice. The research method was a laboratory experiment using a completely randomized design. A total of 24 mice were divided into six groups with four replicates. The groups consisted of a normal control, a diabetic control, a drug control, a 100 mg/kg body weight extract dose, a 200 mg/kg body weight extract dose, and a 400 mg/kg body weight extract dose. The results showed that administration of Bhee fruit extract could lower blood glucose levels, particularly at the 400 mg/kg body weight dose.

Keywords: ***Herbal_Plant, Streptozotocin, Hyperglycemia, Diabetes***

INTRODUCTION

Diabetes mellitus is a non-communicable metabolic disease that is deadly. The prevalence of diabetes is increasing globally and nationally. According to the International Diabetes Federation (IDF), in 2021, it is estimated that more than 537 million people will have diabetes, with projections increasing to 634 million in 2030 and 783 million in 2045. By 2024, the number of adults (aged 20–79 years) with diabetes is projected to reach 589 million. The global prevalence of diabetes (aged 20–79 years) stands at 11.1% and is projected to rise to 13% by 2050 (IDF, 2025). The most common type of diabetes is type 2 diabetes (Abid, 2024). Factors contributing to the increase in diabetes prevalence include changes in lifestyle, dietary patterns, and rapid urbanization (Mohan, 2023). Insulin resistance in Indonesia's urban population has increased significantly due to unbalanced diets and lack of physical activity (Kurniawan et al., 2022).

Indonesia ranks seventh globally with the highest number of diabetes patients (Oktora, 2022). The prevalence of diabetes in Indonesia reaches 10.9% (approximately 21 million people), with only 25% diagnosed (Kemkes R1, 2018). In 2023, the prevalence of diabetes reached 11.7%, an increase from 5.7% compared to 2007 (Afifah, 2022). The prevalence of diabetes in Aceh Province, based on blood sugar tests (≥200 mg/dl), reached 10.9% (Sufyan, 2023). The prevalence of diabetes in Aceh Province ranks seventh nationally (2022). The West Aceh Health Office recorded 3,533 diabetes patients in 2020. The estimated number of diabetes patients surveyed by the West Aceh Health Office surveillance officers was 4,325.

Controlling blood glucose levels in diabetic patients is necessary to prevent serious complications (Fortuna, 2023) using both synthetic chemical drugs and herbal remedies. The use of herbal remedies as antidiabetic agents is increasing because they are considered to have minimal side effects, are easily accessible, and are more affordable. Indonesia has approximately 7,000 types of plants with medicinal potential (Anugerah, 2022). The presence of bioactive compounds and antioxidants is why herbal plants are used as raw medication materials (Pavlić, 2023). The inventory of herbal plants as raw materials for antidiabetic medications remains limited in West Aceh. The Bhee fruit (Melastoma Sp) is a plant with potential as an antidiabetic agent. It is abundant but has not been widely utilized.

Research has proven that the fruit has the potential to be an antidiabetic agent. Ethanol extract of Bhee fruit can reduce glucose levels in alloxan-induced zebrafish (Novitalia, 2022) and glucose levels in alloxan-induced diabetic mice (Rinawati, 2023). Bhee fruit vinegar can lower glucose levels in diabetic mice (Fitria, 2023). Previous research demonstrated that an infusion of Bhee fruit powder can also lower blood glucose levels in alloxan-induced diabetic mice. As a diabetogenic agent, Alloxan is considered less effective than streptozotocin (STZ). Alloxan is unstable and has side effects such as nephrotoxicity and hepatotoxicity, while STZ is more selective toward pancreatic β cells, resulting in fewer side effects (Pratiwi, 2022). This study is important for empirically testing the potential of Bhee fruit as an antidiabetic agent.

METHOD

This study was conducted at the UNIMED Faculty of Medicine Laboratory for testing extracts on laboratory animals and at the UNIMED Histology Laboratory for histological observation of the pancreas. The research design used a completely randomized design with six treatments and four replicates. A total of 24 mice were obtained from Federer's formula calculation. The treatment groups in this study were as follows:

KN = Mice fed only food and distilled water.
KD = Mice conditioned for diabetes without administration of the extract.
KO = Diabetic mice + metformin 1.3 mg/ kg body weight as a control
P1 = Diabetic mice + Bhee fruit water extract at a dose of 100 mg/ kg body weight
P2 = Diabetic mice + Bhee fruit water extract at a dose of 200 mg/kg body weight
P3 = Diabetic mice + Bhee fruit water extract at a dose of 400 mg/kg body weight

Research Procedure

Bhee fruit was collected from the western region of Aceh, and only ripe and fresh fruits were selected. The selected fruits must be thoroughly cleaned of debris and immediately dried in an oven at 60–80°C. The dried fruits were ground using a blender and then sieved to obtain the desired powder. The powder was macerated using water as a solvent for 2×24 hours.

Diabetic Animal Model Preparation

The animals used were male mice obtained from UNIMED, which were acclimatized at room temperature for 7 days. STZ induction was performed using a 30 mg/kg body weight dose dissolved in citrate buffer pH 4.5. STZ was injected intraperitoneally for five consecutive days to induce hyperglycemia in the mice.

Preparation of Metformin Solution

The metformin dose used in humans is 500 mg/kg body weight. In this study, the Laurence-Bacharach formula was used to determine the dose for mice, resulting in the following: 500 x 0.0026 = 1.3 mg/kg body weight. Ten metformin tablets were weighed, and the average weight per tablet was calculated and then converted to the mouse dose of 1 ml/20 g. The metformin tablets were placed in a mortar and ground into a fine powder, then 1% Na CMC 50 ml was added gradually while grinding until homogeneous (Aziz, 2021).

Testing of Bhee Fruit Water Extract on Mice

The extract testing was conducted after the mice developed diabetes when the blood glucose measurement reached >200 mg/dl. The extract was administered in vivo using a syringe needle at 100, 200, and 400 mg/kg BW doses for 28 days.

Blood Glucose Measurement

The glucose level measured was fasting glucose. Before the glucose measurement process, the mice were fed 50% of their usual diet the night before fasting. The device used was an Easy Touch Glucometer test. The blood collection procedure followed the research by Sasmita (2024). Glucose levels were measured before and after STZ induction on days 7, 14, 21, and 28.

Data Analysis

Blood glucose levels were analyzed descriptively in tables and graphs. Data processing and analysis were performed using computer software. For blood glucose measurements in each group, an ANOVA analysis was conducted. Further analysis was performed using the Duncan Multiple Range Test (DMRT) if significant differences were found between treatment groups

RESULTS AND DISCUSSION

This study was conducted for approximately one month to analyze the effectiveness of Bhee fruit (Melastoma Sp) water extract on blood glucose levels in Streptozotocin (STZ)-induced diabetic mice. The first blood glucose measurement was performed after the mice had undergone an acclimatization period. Acclimatization in mice enhances tolerance and reduces physiological stress (Hermawati, 2020) caused by transportation stress or environmental changes, which can disrupt the cardiovascular, endocrine, and immune systems (Hatriyani, 2024). Blood collection was performed after the mice were fasted for approximately 12 hours (Xu et al., 2021) to ensure that glucose level measurements were not influenced by recently consumed food (Kleiman, 2023). STZ induction was performed on the KO, KD, KN, P1, P2, and P3 mouse groups.

Glucose levels were measured to ensure that glucose levels exceeded 200 mg/kg body weight. The normal range of blood glucose levels in mice is 62.8 mg/dl to 176 mg/dl (Faswita, 2024). Fasting blood glucose levels (FBG) in diabetic mice range from ≥ 135 mg/dL (King et al., 2020), though other references indicate that fasting glucose levels in mice can reach 200 mg/dL. This data serves as a baseline for comparing changes that occur after STZ induction, thereby providing a clear picture of the effects of the treatment administered. The increase in fasting blood glucose levels in mice before and after STZ induction in this study is shown in the following table:

Table 1. Changes in glucose levels before and after STZ induction

Treatment Group	Blood Glucose Level	
	Before STZ Induction	After STZ Induction
KN	92.75±6.55[a]	97±4.24[a]
KO	94.5±4.51 [a]	241.5±3.11[b]
KD	97±5.48 [a]	256.25±5.06[c]
P1	93.75±5.74 [a]	256±4.55 [c]
P2	96.5±6.24 [a]	251.25±5.97 [c]
P3	98.25±8.66 [a]	250.25±6.13 [c]

Based on the table above, the mice's average blood glucose levels before induction were all within the normal range, ranging from 92.75 to 97 mg/dL. There were no significant differences in blood glucose levels among the groups. STZ significantly influenced changes in blood glucose levels in mice with induced diabetes. Blood glucose levels in STZ-induced mice increased to 241.5–256.25 mg/dL, significantly different from the normal control group. Statistical analysis assessed the significance of changes in blood glucose levels. To ensure the data were normally distributed, the Shapiro-Wilk test was performed. The analysis results showed that the data were normally distributed ($p > 0.05$) in all treatment groups. Levene's homogeneity test was performed to determine whether the data were homogeneous. The test results showed homogeneity of variance between groups ($p > 0.05$). Normality and homogeneity of data are prerequisites for performing an ANOVA test. The one-way ANOVA and Duncan's test results showed significant differences between treatment groups ($p < 0.05$), particularly between the normal control group and the STZ-induced group. The post hoc analysis showed significant differences in glucose levels among the STZ-induced groups ($p > 0.05$), indicating that STZ affects blood glucose levels in diabetic mice. The percentage increase in blood glucose levels after STZ induction is shown in the figure below:

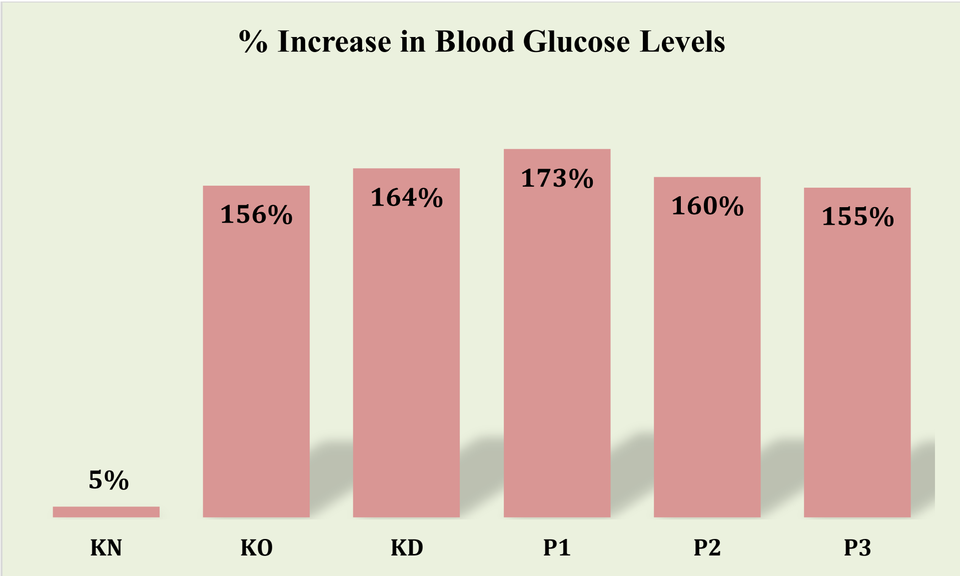

Figure 1: Average percentage increase in blood glucose levels in mice after STZ induction

The KN group experienced a 5% increase, but its glucose levels remained within the normal range due to stable glucose homeostasis unaffected by STZ or other treatments. This condition indicates that the role of pancreatic β cells in the Normal Control group is functioning properly. Although blood glucose levels differed among groups, the percentage increase in all diabetic-conditioned mouse groups reached >100%. The highest percentage increase in glucose levels was observed in the P1 group, reaching 173%. The effects of STZ induction may vary among mice, potentially influenced by other factors such as physiological factors and stress (Sharma, 2023). After confirming that the STZ-induced mice had diabetes, they were administered Bhee fruit water extract for 28 days. Measurement results showed varying blood glucose level reduction patterns across each treatment group. Observations were conducted weekly to monitor the extract's effectiveness in lowering blood glucose levels in diabetic mice. Blood glucose level measurement results are presented in the following table:

Table 2. Average decrease in glucose levels after administration of the fruit extract (Melastoma sp)

Treatment Group	Average Blood Glucose Level (mg/dL)				
	After STZ Induction	Week 1	Week 2	Week 3	Week 4
KN	97±4.24[a]	107±5.10[a]	98.25±5.62[a]	100.25±5.80[a]	101.5±5.32[a]
KO	241.5±3.11[b]	243.5±4.43[c]	186.25±4.35[b]	153.5±5.51[b]	128±4.97[b]
KD	256.25±5.06[c]	240.5±4.51[c]	235.25±6.70[d]	243±6.78[de]	263.75±6.29[f]
P1	256±4.55 [c]	263.25±6.99[d]	261±6.73[e]	251.25±4.57[e]	214.25±4.35[e]
P2	251.25±5.97 [c]	236.25±9.95[c]	238.25±5.38[d]	236.75±6.85[d]	190±6.06[d]
P3	250.25±6.13 [c]	225±4.16[b]	214.75±4.57[c]	198±5.72[c]	171.25±6.29[c]

Statistical analysis of glucose level reduction data showed significant differences between observation times ($p < 0.05$) and between treatment groups ($p < 0.05$). Duncan's post-hoc test showed that starting from the second week, there were significant differences between all treatment groups and the diabetic control group. The pattern of glucose level reduction showed a linear trend and was dose-dependent. Blood glucose measurements in the first week showed that the KN group remained in the normal category. Blood glucose levels in the diabetic-conditioned mouse group remained high, with an average exceeding 200 mg/dL. Blood glucose levels in the P1 group were higher than in the other groups, reaching 263.25 mg/dL. The lowest blood glucose levels were observed in the P3 group. Blood glucose levels in the KO, KD, and P3 groups were not significantly different. Blood glucose levels on day 7 were not yet stable, which may be due to the ongoing effects of STZ on the mice's metabolism. Measurements in the second week showed the beginning of differences in blood glucose levels among the treatment groups. The KO group observed the greatest decrease, with blood glucose levels reaching 186.25 mg/dL. Among the groups given the extract, the highest decrease in blood glucose levels occurred in the P3 group (214.75), although the value was higher than that of the KO group. The effect of the extract administration began to be evident. These results indicate that the treatment effect of the Bhee fruit extract began to be evident at the highest dose (P3). In contrast, the low (P1) and medium (P2) doses were ineffective in lowering blood glucose levels. Blood glucose level measurements in the third week began to show significant differences among the treatment groups. Blood glucose levels in the KD group continued to rise. Blood glucose levels in the P2 and P1 groups remained high and were not significantly different from the KD group. The most significant decrease occurred in the KO group, as their blood glucose levels had reached normal levels (153.5 mg/dL), but this decrease was still significantly different from those in the KN group. Blood glucose measurements in the fourth week showed increasing variation. There were differences among the groups. The highest decrease in blood glucose levels still occurred in the KO group. In the extract treatment groups, the highest decrease occurred in the P3 group, which had reached the normal range (171.25 mg/dL). Blood glucose levels in the KD group remained high at 263.75 mg/dL. The normal control group (KN) remained stable. To determine the effect of extract administration on blood glucose level reduction, the overall percentage from day 7 to day 28 must be calculated. The percentage is shown in the following graph:

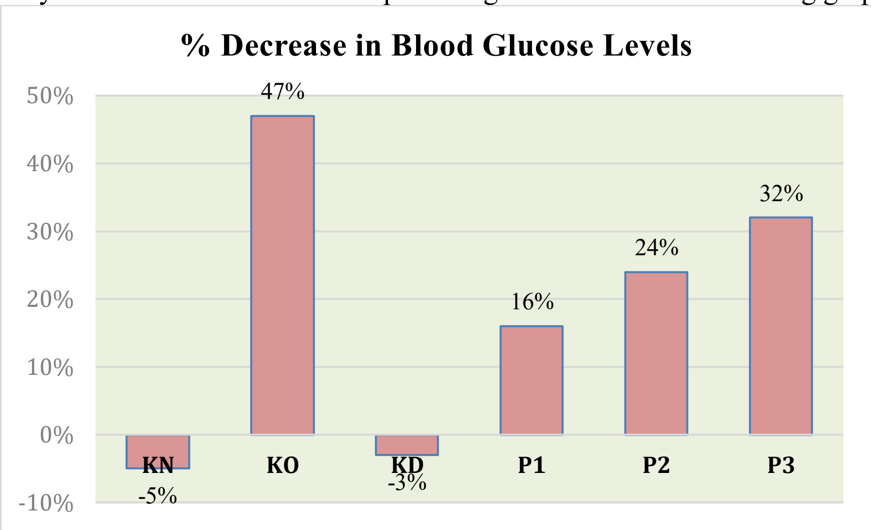

Figure 2. Decrease in glucose levels after administration of Bhee fruit extract (Melastoma Sp)

Based on the graph above, the highest percentage decrease was observed in the KO group, reaching 47%. The highest percentage decrease in glucose levels was observed in the P3 group, reaching 32%. Although the 400 mg/kg BW dose in the P3 group lowered blood glucose levels by the fourth week, the effect of metformin administration in the KO group was not yet matched. Glucose levels in the KD group continued to increase. The percentage decrease in the KD group was -3%. This demonstrates that pancreatic damage caused by STZ induction continues to increase blood glucose levels. STZ successfully induced hyperglycemia in the diabetic animal model. STZ induction selectively affects pancreatic β-cell damage through DNA alkylation, increased ROS levels, and GLUT2 inhibition. DNA alkylation causes DNA fragmentation and PARP activation to repair the DNA damage. PARP-1 consumes NAD^+, which serves as a substrate for ADP-ribose polymerization. NAD^+ functions as a precursor for energy production (ATP). Massive NAD^+ consumption leads to energy depletion and necrosis of pancreatic β cells (Cardinal, 2021).

STZ binds to GLUT-2, facilitating its transport into the cytoplasm of pancreatic β cells. This process triggers mitochondrial depolarization caused by increased Ca2+ ion influx, followed by excessive energy consumption. As a result, energy deficiency occurs within the cell, disrupting insulin production. This insulin deficiency impairs the body's ability to process glucose optimally, leading to elevated blood glucose levels. The effect of STZ on pancreatic β cells also causes changes in the characteristics of insulin in the blood, leading to a decrease in insulin levels and changes in blood glucose characteristics that trigger hyperglycemia. Additionally, the sensitivity of peripheral insulin receptors in STZ-induced rats decreases, contributing to increased insulin resistance (Faida, 2020).

Metformin is one of the drugs used by people with diabetes to control blood glucose levels (Wojeck, 2023). Metformin controls blood glucose levels by activating AMPK in the liver, increasing insulin signaling in muscles and fat, and modulating gut microbiota. AMPK is the central regulator of glucose and fat metabolism. When AMPK is activated by metformin, it suppresses glucose production in the liver and increases peripheral glucose uptake (Hasanvand, 2022). Enhanced glucose uptake signaling by muscle and adipose tissue occurs through the activation of GLUT4. This insulin-dependent glucose transporter is critical in glucose uptake into muscle, fat, and heart tissues. This increased signaling reduces insulin resistance by lowering free fatty acids and inflammation. This mechanism facilitates glucose entry into cells, stabilizing blood glucose levels (Chadt et al., 2020). Metformin also plays a role in intestinal glucose absorption by altering gut microbiota composition. Metformin increases bacteria that produce short-chain fatty acids (SCFAs). If the microbiota producing short-chain fatty acids increases, there will be an increase in the production of butyrate and propionate, which will reduce intestinal inflammation. The mucus barrier in the intestine will also increase, preventing the translocation of pathogenic bacteria (Eun et al., 2022). Phytochemical screening results indicate that the active compounds in the fruit include alkaloids, terpenes, flavonoids, and tannins. These compounds can act as antidiabetic agents capable of addressing hyperglycemia in mice. Tannins function as astringents or chelating agents that constrict the intestinal epithelial membrane, reducing nutrient absorption. Reduced sugar absorption results in a slower increase in blood sugar levels (Ayudia, 2023). Tannins act as inhibitors of intestinal α-amylase and α-glucosidase, enzymes in carbohydrate digestion (Li et al., 2022). The inhibition of these digestive enzymes forms hydrogen bonds with the active sites of the enzymes, leading to a conformational change that inhibits their catalytic activity. This inhibition causes a decrease in the conversion of starch into glucose and reduces glucose absorption in the intestines (Saini et al., 2023). Tannins also increase food viscosity, thereby slowing the rate of glucose absorption. Tannins can improve insulin signaling by inhibiting NF-κB and TNF-α (Chadt, 2020).

Flavonoids act as antioxidants that can capture free radicals that cause damage to pancreatic β cells and inhibit damage to pancreatic β cells so that the remaining β cells continue to function. These antioxidants are believed to protect several normal β cells, thereby enabling the regeneration of existing β cells through mitosis (Ananda, 2024). Flavonoid compounds can act as inhibitors of alpha-amylase and alpha-glucosidase, which can repair and prevent cell damage caused by oxidative events due to free radicals (Selvia, 2020). They have antioxidant activity by capturing or neutralizing free radicals associated with phenolic OH groups. Additionally, flavonoids also have a mechanism to lower blood glucose levels by increasing insulin secretion (Hidayati, 2022). Flavonoids can open K^+-ATP channels, depolarizing the membrane and increasing insulin secretion. Another mechanism involves activating AMPK to enhance GLUT4 translocation in muscles and fat tissues while reducing phosphoenolpyruvate carboxykinase (PEPCK) in the liver, thereby decreasing gluconeogenesis (Chen et al., 2016).

The mechanism by which alkaloids lower blood glucose levels is by inhibiting the α-glucosidase enzyme in the duodenal mucosa, thereby hindering the breakdown of polysaccharides into monosaccharides. The release of glucose is also slower, and its absorption into the blood is less rapid and lower, thereby avoiding a spike in blood glucose levels (Singh et al., 2022). Each of these bioactive components plays an important role in lowering blood glucose levels. However, there are dominant roles for each bioactive component, including tannins playing a greater role in lowering postprandial glucose (after meals) through the inhibition of α-amylase to reduce the conversion of starch into glucose. Flavonoids focus on multi-target modulation in the pancreas, liver, and peripheral tissues, while alkaloids exhibit strong pharmacological properties in pancreatic β cells and insulin signaling (Muhammad et al., 2021).

CONCLUSION

Bhee fruit water extract lowered blood glucose levels in STZ-induced diabetic mice at 100, 200, and 400 mg/kg BW doses. The highest percentage of blood glucose reduction was observed in the group of mice given the 400 mg/kg BW dose.

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