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The Efficacy of Green Grapefruit (*Vitis Vinifera* L) Extracts on Reducing Blood Glucose in a Diabetic Rat Model

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Abstract: Diabetes mellitus (DM) is a chronic disease in which the pancreas cannot produce enough insulin hormone or when the body cannot use the insulin hormones properly. Green grapes contain flavonoids, anthocyanins, tannins, phenolic acids, and resveratrol and are high in antioxidants that are beneficial in lowering blood glucose levels. This study aims to determine the effectiveness of green grapefruit (*Vitis vinifera L*) extract in reducing blood glucose levels in a diabetic rat model. This research is a true experiment with a post-test-only control group design, using 24 male Wistar white rats aged 8-12 weeks and weighing 130-200 grams. The rats were divided into three groups: the positive control (I), green grapefruit extract (II), and normal group (III). The results showed that green grapefruit extract effectively reduced blood glucose levels in experimental animals by a percentage of 98.4%. The Independent T-Test showed a value of ρ>0.05 (0.533), so there was no significant difference in glucose levels between green grapefruit extract and positive control. This study concludes that green grapefruit extract was effective at 98.4% in reducing blood glucose levels in the diabetic rat model, compared to the positive control. The promising results of this study need to be continued by establishing a standardized optimal dose, assessing long-term effects, and implementing clinical trials to evaluate efficacy and safety.
Keywords: Blood glucose; diabetic; extract grapefruit
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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease in which the pancreas cannot produce sufficient amounts of insulin or when the body cannot use the insulin hormone (World Health Organization, 2016). The global data estimates a staggering increase in the number of type 2 DM patients in Indonesia, from 8.4 million in 2000 to a projected 21.3 million in 2030. This alarming trend is further highlighted by the International Diabetes Federation (IDF), which ranks Indonesia sixth with 10.3 million DM patients. The Basic Health Research report also reveals a significant rise in DM prevalence to 8.5%, affecting around 20.4 million people. Bali, one of Indonesia's provinces, is particularly hard hit, with a DM prevalence of 1.5% (Riskesdas, 2018). Diabetes mellitus, known as the 'mother of diseases', can affect every organ of the body and cause various symptoms.

Diabetes mellitus (DM) is a syndrome characterized by hyperglycemia conditions that, over time, increase the risk of damage to the kidneys, eyes, and nerves, especially the heart, as well as medium and large blood vessels (Noor, 2015). Metabolic syndrome and hyperglycemia are closely related to DM disease,

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characterized by a blood glucose level ≥200 mg/dl and a fasting blood glucose level ≥126 mg/dl (Yuniarti et al., 2018).

There are two types of DM: type 1 DM and type 2 DM. Type 1 DM occurs when the body cannot produce sufficient insulin hormone. This condition usually occurs in children and adolescents due to abnormalities in pancreatic cells. The combination of insulin resistance causes type 2 DM, mainly caused by obesity and lack of insulin secretion (Widowati, 2008). Type 2 DM disease is usually in elderly patients (<50 years old) (Soelistijo, 2021). Over 95% of DM diseases worldwide are type 2 DM (World Health Organization, 2023).

Hyperglycemia is the main factor that leads to the development of complications in patients with type 2 DM. Hyperglycemia conditions can cause damage to DNA, lipids, and proteins. The level of damage is related to the production level of hyperglycemic-induced reactive oxygen species (ROS) free radicals due to oxidative stress. Hyperglycemia conditions can cause inflammation by continuously producing excessive free radicals and reduced antioxidants (Oguntibeju, 2019). This condition will trigger a non-specific immune response to activate macrophages and produce pro-inflammatory cytokines, such as IL-8, IL-6, IL-1, and TNF. These cytokines can cause insulin resistance in type 2 DM disease (Yuniarti et al., 2018).

Antioxidants are a group of compounds that can protect cells from damage caused by free radicals. Antioxidants are an efficient way to counteract oxidative stress. Antioxidants can inhibit free radicals through the process of redox reactions. Oxidation reactions in the body can generate free radicals, creating a chain reaction that leads to cell damage. Antioxidants can terminate this chain reaction by eliminating free radical intermediates and suppressing further oxidation reactions by oxidizing themselves. Under excessive oxidative stress, the typical human antioxidant system is unable to neutralize the destructive effects of free radicals, which can damage cellular DNA, lipids, proteins, and other macromolecules, resulting in the development of chronic illnesses and premature aging. Hence, the body requires exogenous antioxidants to scavenge free radicals and prevent their adverse effects to protect cells and tissues (Ardani et al., 2023; Hamid et al., 2010; Nimse & Pal, 2015).

Natural antioxidants from plant sources are more acceptable, reliable, and safer to promote health and prevent the adverse effects of free radicals. Previous research has proven the presence of bioactive compounds with various pharmacological properties, including antioxidants, in a variety of natural products, such as garlic, lemon, cemcem leaves, and Gamal leaves (Artaningsih et al., 2018; Bekti et al., 2022; Vinenthy et al., 2019). Grapes are another natural substance with antioxidant properties due to their high polyphenol and anthocyanin content (Arwati et al., 2022). Green grapes (Vitis vinifera L) contain over 1600 phytonutrients, including numerous types of flavonoid components such as catechins (catechins and epicatechins), anthocyanins (peonidin, cyanidin, and malvidin), flavonols (quercetin, kaempferol, and isorhamnetin), and resveratrol (Almomen et al., 2017). Grapes have higher antioxidant levels than other plants (Dewi et al., 2023). Green grapes' anti-inflammatory properties can help reduce inflammation associated with type 2 diabetes (Rasines-Perea & Teissedre, 2017).

Due to their high vitamin C content, green grape seeds and peels provide a high concentration of antioxidants (Mikhael & Soegihardjo, 2013). Research findings by Hassan & Hassan, 2010 suggest that green grape seed extract can effectively scavenge the reactive oxygen species (ROS). Furthermore, green grape seed extract has been shown to lower blood glucose levels in DM model rats. Green grape seeds contain various bioactive substances, including flavonoids, catechins, and the

Oligomer Proanthocyanidin Complex (OPC) (Djoka et al., 2012). However, the potential of green grapefruits to maintain glucose levels in rat models has not been widely studied. In the face of the increasing prevalence of type 2 diabetes, identifying substances that can scavenge free radicals or regulate blood glucose levels is of utmost importance. Therefore, this study will compare the efficacy of green grapefruit extract in reducing blood glucose levels in the diabetic rat model to that of the positive control, underscoring the significance of the study in the context of the rising prevalence of type 2 diabetes.

MATERIALS AND METHODS

Materials

The materials of this study were a total of 5 kg of local green grapefruits obtained from Singaraja, Bali, Indonesia. The other materials were sodium carboxymethyl cellulose (Sigma-Aldrich), glibenclamide 5mg, Streptozotocin (STZ) [2-deoxy-2-(3-methyl-3-nitrosurea) 1-D-glucopyranose] (Merck-Millipore), blood glucose standar and reagent (ELITechGroup), ethanol 96% and aqua dest. The tools were a photometer (ELITechGroup, type of Microlab 1000), centrifuge (Gemmy), analytical balance (Radwag, Poland), refrigerator, and rotavapor (Buchi R-300).

Methods

Research design

The type of research is a true experiment with a post-test-only control group design. The study was carried out on August 2023 and February 2024 at the Bio Mice and Rat Laboratory in Denpasar and also conducted in the Clinical Chemistry Laboratory of the Department of Medical Laboratory Technology, Health Polytechnic of Denpasar, Bali. The experimental animals were male Wistar white rats aged 8-12 weeks and weighing 130-200 grams. We used simple random sampling approaches to select male rats that met the inclusion criteria, demonstrating the care taken in our research methodology. The Federer formula was applied to calculate the sample size, and 24 rats were chosen for this study. The rats were divided into three groups: the positive control (I), green grapefruit extract (II), and normal group (III). By using the following formula, the percentage of decreasing blood glucose levels was determined (Equation 1):

$$\frac{The\ average\ of\ reducing\ blood\ glucose\ levels\ of\ the\ treatment\ group}{The\ average\ of\ reducing\ blood\ glucose\ levels\ of\ the\ positive\ control\ group} x100\%$$

Furthermore, the obtained data was recorded, processed, and presented as narratives and tables. It is important to note that our study was conducted with the utmost respect for ethical standards, and we obtained the necessary approval from the Ethics Commission of Health Polytechnic of Denpasar, DP.04.02/F.XXXII.25/0776/2023.

Preparation of green grapefruit (Vitis vinifera L) extract

The green grapefruits used in this study were obtained from Singaraja, Bali, Indonesia. Green grapefruit extract was prepared according to the study's method (Habibah et al., 2023). A total of 5 kg of local green grapefruits were washed thoroughly with running water and then dried by aerating to remove the remaining washing water. Next, the green grapefruits were cut into several pieces to speed up the drying process. Then, the green grapefruits were dried using an oven at 45˚C until completely dry. After drying, the grapefruit samples were sorted again to separate other parts included in the drying process. The dried green grapes were then crushed using a blender and sieved to obtain simplisia powder of relatively the same size.

Furthermore, the powdered simplisia is stored in a closed and airtight jar at room temperature, ensuring its preservation and quality.

Furthermore, 200 grams of simplisia powder was extracted by maceration method in 96% ethanol solvent with a ratio of 1:5. The maceration process was repeated twice to increase the effectiveness of the extraction process. In addition, agitation was carried out for 15 minutes daily so that ethanol could reach all parts of the leaf powder. The extract obtained was filtered with filter paper. The filtrate obtained was concentrated in a rotary evaporator at 60°C. The extract yield based on the simplisia powder's mass was 10.4%.

Acclimation of experimental animals

The acclimation of the experimental animals in this study was carried out carefully, following the method in the study (Naibaho et al., 2020) with modifications. Twenty-eight male Wistar rats aged 8–12 weeks weighing 130–200 grams were randomly divided into four treatment groups. Each treatment group consisted of seven experimental animals. All the experimental animals were carefully adapted to their new environment and given standard feed for seven days, ensuring their readiness for the study. This careful adaptation process was designed to respect the animals' well-being and ensure their comfort during the study.

Streptozotocin (STZ) Administration

The STZ administration was carried out on the eighth day of acclimation. The purpose of this administration is to induce diabetes in the rats. A single-dose injection of STZ was done intraperitoneally at a dose of 60 mg/kg BW. Furthermore, the experimental animals were incubated for five days before the subsequent treatment to allow the STZ to take effect and the animals to stabilize (Husna et al., 2019).

Preparation of Test and Control Solution

The test solution in green grapefruit extract was given to the experimental animals at 200 mg / kgBB. The dosing was carefully calculated using the following equation, ensuring the accuracy and precision of the experiment. This precise dosing calculation is a testament to our experiment's scientific rigor, enhancing our results' reliability (Equation 2).

$$\text{Weight of extract} = Dose\ \left(\frac{mg}{kg}\right) \times Experimental\ animal\ weight\ (g)$$

The positive control solution was used glibenclamide. The therapeutic dose of glibenclamide in 50 kg humans is 5mg/kg BW, and the dose conversion value for rats is 0.018. Furthermore, the glibenclamide was administrated to the experimental animals with the following equation (Equation 3) (Naibaho et al., 2020).

$$Dose\ \times Weight\ of\ experimental\ test\ (kg) = Glibenclamide\ concentration\ (\frac{mg}{mL}) \times V(mL)$$

The green grapefruit extract test solution and the glibenclamide positive control solution were dissolved in 0.5% Na-CMC. This dissolution process was carefully carried out to ensure the solutions' suitability for administration to the experimental animals.

Blood Glucose Examination

The blood glucose levels were measured spectrophotometrically with great care and attention to detail. The process was initiated before treatment, specifically on day 8 of acclimation, and then repeated after treatment, on day 6 after STZ injection, and on day seven after extract administration. The blood was drawn after the animals were anesthetized, ensuring their comfort and safety. The blood samples were collected by scraping one end of the microhematocrit into the eye's medial canthus or orbital sinus, under the eyeball and toward the optic foramen, and the other end into

the mouth of the blood collection tube. The microhematocrit was then rotated until it injured the plexus, then returned four times until approximately 1.5 mL of blood was obtained from each experimental animal. The blood obtained was collected into an anticoagulant-free tube and allowed to stand at room temperature for two hours. Then, the blood was centrifuged at 3000rpm for 5 minutes to obtain the serum. The serum obtained from each experimental animal is approximately 0.5 mL, sufficient for photometric examination of glucose levels.

Measuring blood glucose levels began with the conditioning of reagents and samples at room temperature. Then 3 test tubes were prepared: one for blank (500 µL reagent), one for standard (500 µL reagent and 5 µL standard), and one for sample (500 µL reagent and 5 µL sample). The solution mixture was homogenized and incubated at room temperature for 10 minutes. Subsequently, the absorbance of the blank and standard solutions and the blood glucose level of the sample were measured at a wavelength of 505 nm. The blood glucose levels of experimental animals were expressed in mg/dL and declared normal if they were 50-135 mg/dL (Hidayaturrahmah et al., 2020).

The Efficacy Determination

The efficacy testing began on the eighth day after acclimation by measuring body weight and blood glucose levels. Blood glucose levels were measured before STZ injection to determine the animals' initial condition. Then, blood glucose levels were measured again on the sixth day after the STZ injection to determine the condition of hyperglycemia in experimental animals. The measurement process involved a careful and precise procedure. Experimental animals are declared hyperglycemia if their blood glucose levels are≥ 200 mg/dL (Nofianti et al., 2020). The efficacy of green grapefruit extract is known by measuring blood glucose levels on the seventh day of treatment.

Groups I and II animals were given a single dose of STZ intraperitoneally at 60 mg/kg, while group III was not subjected to STZ treatment. All groups were then incubated for five days. On the sixth day post-STZ administration, blood glucose levels were measured to confirm the onset of hyperglycemia in groups I and II. Once the hyperglycemia condition was established, the following treatments were administered for seven days: positive control (I), green grapefruit extract (II), and the normal group (III). Group I received glibenclamide at a dose of 0.09 mg/kg BW/day, group II was given green grapefruit extract at 200 mg/kg BW/day, and group III was provided with standard feed. The suspensions of green grapefruit extract and glibenclamide were administered per-orally using an oral sonde with meticulous care and precision. The experimental animals were held and positioned straight. The sonde was inserted slowly and gently into the esophagus before slowly injecting the green grapefruit extract and glibenclamide suspension. During the study, on the seventh day of treatment, there were 3 rats died (drop out, DO); there were 2 in the positive control group and 1 in the green grapefruit extract group.

The efficacy of green grapefruit extract was determined by comparing the average decrease in blood glucose levels of experimental animals in the extract group with the positive control group using Equation 1. The data on the decrease in blood glucose levels were then statistically tested using the Independent T-test to determine the difference between the extract and positive control groups.

RESULTS AND DISCUSSION

Extraction and Grapefruits Extract

Green grapefruit extract was prepared by macerating 200 grams of simplicia powder in 1L of 96% ethanol. The samples were ground into powder to improve the surface area and, thus, the efficacy of the extraction procedure. This results in a higher yield of extract collected. Proper particle size promotes a smooth and effective extraction process that takes a short time because the contact area between the material and solvent rises with particle size (Ningsih et al., 2019).

In this study, the simplicia powder was extracted using the maceration method. The maceration method has numerous advantages and is particularly useful for separating chemicals from natural sources. During the soaking process, the cell wall breaks due to the pressure difference between the inside and outside of the cell. This enables the chemicals in the cytoplasm to dissolve into the solvent. This extraction method can be optimized by adjusting the soaking time. The maceration process has several advantages, such as not requiring fine powder, requiring no specific abilities, and less distilled loss (Atun, 2014).

The solvent for this maceration procedure is 96% ethanol. Ethanol is a polar solvent, which allows it to extract polar chemicals, such as phenolic compounds and flavonoids. This solvent is used due to its neutrality, selectivity, low toxicity, ability to dissolve a wide range of secondary metabolites, and rapid evaporation. Following the maceration process, the filtrate is separated from the residue. This evaporation procedure is critical for future research since it reduces matrix intervention, particularly from the solvent used (Fauziyah et al., 2022; Habibah et al., 2023; Riwanti et al., 2020).

The maceration procedure was performed twice to improve extraction efficiency. In addition, agitation was performed for 15 minutes daily to ensure that ethanol reached all of the green grapefruit powder. The extract was filtered using filter paper. The filtrate was concentrated in a vacuum rotary evaporator at 60°C. The concentrated extract achieved in this process is 20.8 grams, indicating a yield of 10.4%. The extract yield is calculated to determine the ratio of extract obtained to Simplicia's initial weight. Furthermore, extract yield can be utilized qualitatively to evaluate the amount of bioactive chemicals in the extracted material (Utami et al., 2020).

Diabetic Rat Model

The experimental animals in this study were Wistar rats aged 8-12 weeks and weighing 130-200 grams. Rats are the most common animal model in research due to their docile nature, short gestation time and life span, and established health and genetic history. Furthermore, the rat genome is identical to the human genome, allowing manipulation of the mouse genome to generate animal models with characteristics similar to human disorders (Husna et al., 2019).

The development of the diabetic animal model begins with the acclimation of the experimental animals. Acclimation is crucial in animal model research, particularly in diabetic rat models. This process leads to several physiological adaptations, such as alterations in metabolic parameters, hormonal levels, and tissue responses, significantly influencing treatment outcomes. The most significant benefit of acclimation is its role in reducing stress and variability in experimental outcomes. This not only enhances the reliability of research findings but also ensures the well-being of the animals. By minimizing stress responses that can affect the results, acclimation ensures that animals exhibit lower glucose and stress hormone levels during procedures (Marin et al., 2023). A well-structured acclimation protocol can lead to more accurate physiological measurements, as seen in studies where prolonged restraint was managed effectively (Kirby et al., 2023).

The test animals' acclimation step is carried out to allow animals to adapt to their new environment during the treatment process. These adaptations include alterations in metabolic parameters, hormonal levels, and tissue responses, which can affect the efficacy of therapeutic interventions. During the acclimation stage, the test animals will be handled more frequently to become more docile, lowering the likelihood of accidents and stress during treatment (Husna et al., 2019).

In this study, the acclimation stage lasted for seven days. During the acclimation stage, the experimental animals were placed in cages in a room with a temperature of 20-24°C, given standard feed and water, and a 12-hour light-dark cycle. The acclimation stage in this study was successfully conducted. None of the animals appeared weak, stressed, or sick during acclimation. Animals seemed to remain active and willing to eat and drink.

Regarding diabetes, the animals still showed good condition, as evidenced by the pre-treatment blood glucose levels measured on the eighth day of acclimation. The measurement results showed that the initial blood glucose levels before administrating STZ were 51-70 mg/dL. This value is in the normal category because it is still in the range of 50-135 mg/dL.

Each test and control solution was dissolved in 0.5% Na-CMC. This choice of solvent was made because it is commonly used in pharmacological studies, is well-tolerated by rats, and allows for easy administration of the solutions. In order to induce DM non-genetically, STZ was administered intraperitoneally at a single dose of 60 mg/kgBB. 'Non-genetically generated DM animal models' refers to diabetes models induced in animals without altering their genetic makeup. Further treatment was carried out five days after STZ administration. These animal models exhibit a similar pathophysiology to humans. In animal models of diabetes, the STZ is often used as a diabetogenic agent. STZ offers various advantages over other dibetogenic drugs, including greater effectiveness, reproducibility, and solution stability. Animal models of DM induced by STZ administration show similarities in several structural, functional, and biochemical abnormalities of DM disease, making it more suitable as a model to examine the mechanism of DM (Eleazu et al., 2013; Husna et al., 2019; Lee et al., 2010).

The hyperglycemia condition in experimental animals was indicated by elevated blood glucose levels of more than 200 mg/L (Nofianti et al., 2020). Based on the results obtained, it is proven that administering STZ at a single dose intraperitoneally can cause hyperglycemia conditions in experimental animals with an average blood glucose level of 653.3 mg/dL.

STZ is cytotoxic to pancreatic cells, and the effect will appear 72 hours after STZ administration, depending on the dose given. This 72-hour time frame is significant as it marks the onset of the toxic effect of STZ on pancreatic cells. The toxic effect of STZ begins with the intake of STZ into the cell through glucose transporter-2 (GLUT2). The toxic effect of STZ is selective to pancreatic cells because STZ has a glucose group in its structure that makes it easier for STZ to enter pancreatic cells. An increase in experimental animal blood glucose levels indicates the adverse effect of STZ administration on pancreatic cells. STZ can also cause nephrotoxic, hepatotoxic, heart, and fat tissue damage and increased oxidative stress, inflammation, and endothelial dysfunction (Elsner et al., 2000; Husna et al., 2019; Valentovic et al., 2006).

The Efficacy of Grapefruits Extract on Reducing Diabetic Rat Model

The efficacy of green grapefruit extract was determined by comparing the reduction in blood glucose levels between the treatment group and the positive control

group. The blood glucose levels of experimental animals were measured using a spectrophotometer method. Figure 1 shows the measurement findings for blood glucose levels. According to the data presented, experimental animals' blood glucose levels decreased following the administration of green grapefruit extract and the positive control, offering the potential impact of green grapefruit extract on diabetes treatment.

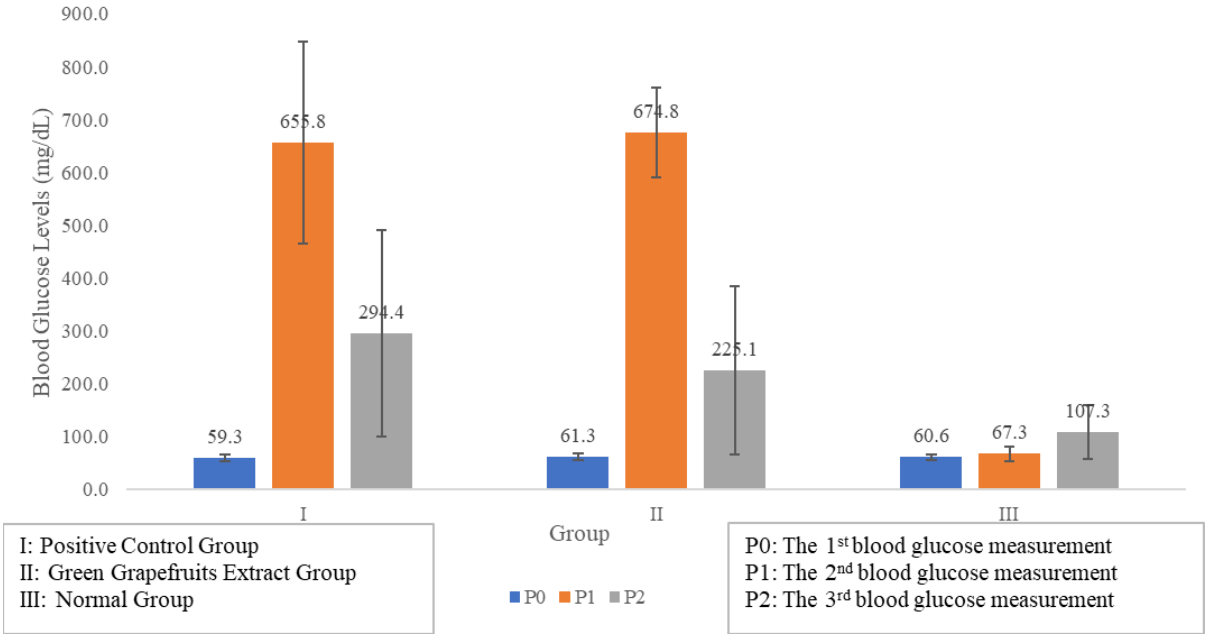

Figure 1. The Average of Blood Glucose Levels

P0 is the blood glucose level of the experimental animals at the first measurement. Blood glucose in P0 was measured before treatment on the eighth day of acclimation. This measurement is done to determine the initial condition of the animals. P1 is the blood glucose level of the animals on the second measurement. Blood glucose in P1 was measured on the 6th day after STZ injection or the 14th day of acclimation. Measurement of blood glucose in groups I and II on day 14 to evaluate the development of diabetes after STZ injection in groups I and II. Diabetes condition in experimental animals is characterized by hyperglycemia with blood glucose levels of more than 200 mg/dL. On the same day, the blood glucose of the normal group was measured. P2 is the blood glucose level of experimental animals on the third measurement. Blood glucose in P2 was measured on the 7th day after treatment or the 21st day of acclimation. This measurement was done to determine the ability of glibenclamide as a positive control and green grapefruit extract to reduce the blood glucose of STZ-induced experimental animals. The usual group's blood glucose level was also compared on the same day.

The efficacy of green grapefruit extract was determined by comparing the reduction in blood glucose levels between the treatment group and the positive control group. The blood glucose levels of experimental animals were measured using a spectrophotometer method. Figure 1 shows the measurement findings for blood glucose levels. According to the data, experimental animals' blood glucose levels decreased following green grapefruit extract and optimistic control administration.

After seven days of treatment, blood glucose levels in the green grapefruit extract group at a dose of 200 mg/kg BW/day reduced from 666.8 mg/dL to 225.1 mg/dL, with an average decrease of 449 mg/dL. Some previous studies have also proven that other parts of grapes, such as the seeds and skins, have antidiabetic abilities. A methanolic grape seed extract administered at 50 mg/kg showed significant reductions in blood glucose and improved lipid profiles in diabetic rats (Elmhdwi et al., 2017). Another study showed that a higher dose of grape seed extract, a dose of 100 mg/kg eff, actively decreased blood glucose levels and improved insulin sensitivity (Ganjali et al., 2012). A 200 mg/kg dosage of Vitis vinifera grape skin extract significantly lowered glycemia and enhanced insulin signaling in diabetic mice (Soares De Moura et al., 2012). Another study found that a single dose of 500 mg/kg of a grape polyphenol-soy protein complex lowered blood glucose levels from 236 mg/dL to 177 mg/dL in diabetic mice (Roopchand et al., 2013).

The presence of bioactive components in green grapefruit extract is linked with its ability to lower blood glucose levels in experimental animals. Green grapes include a variety of flavonoid bioactive components, including flavonols, proanthocyanidins, anthocyanidins, and flavanone molecules, such as naringin and naringenin, which play a role in metabolic processes such as regulating blood glucose(McRae & Kennedy, 2011), (Razavi & Hosseinzadeh, 2019). Most bioflavonoid chemicals in grapes are anthocyanidins, specifically the Oligomer Proanthocyanidin Complex (OPC) (Emilda, 2018).

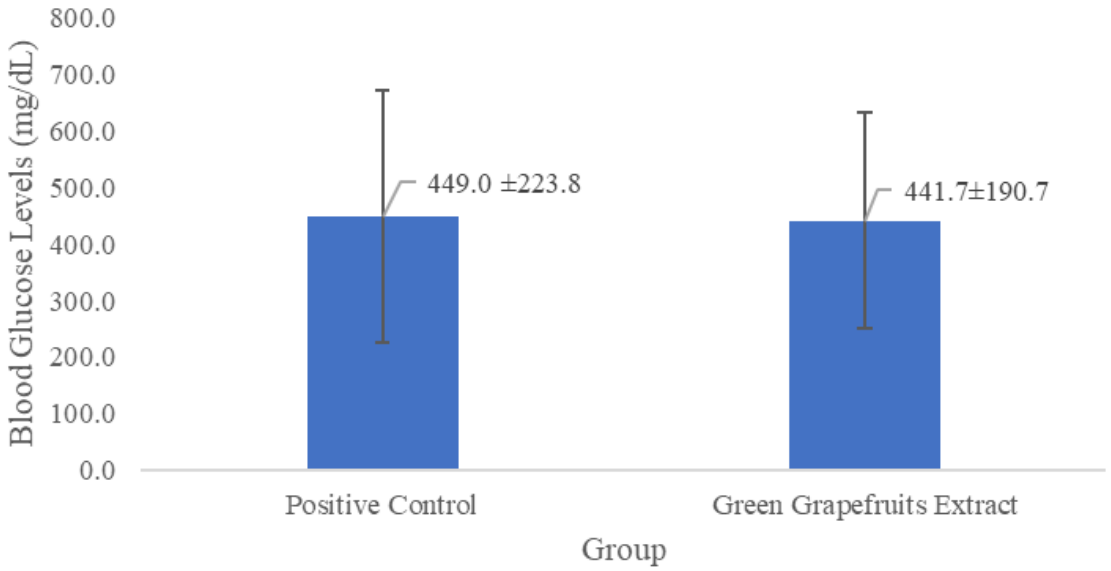

Figure 2. The Average of Reducing Blood Glucose Levels

The OPC molecules have a more potent antioxidant capacity than vitamins C and E; hence, they can reduce hyperglycemia and inflammation (Uzun et al., 2013), (Pandey & Rizvi, 2014). Grapes contain chemicals closely associated with antioxidant activity, cholesterol reduction, and antiobesity effects; therefore, they can potentially prevent metabolic syndrome (Razavi & Hosseinzadeh, 2019).

The ability of grapes to reduce blood glucose levels occurs through several pathways. Grapefruit extract contains several bioactive compounds that can regulate glucose metabolism by decreasing the expression of pro-inflammatory genes in the liver and adipose tissue to reduce blood glucose levels. Additionally, the bioactive

compound of grapefruits has been shown to improve glucose intolerance in diabetic rats by suppressing hepatic gluconeogenesis, as evidenced by increased glucokinase activity and elevated hepatic glycogen-concentration while suppressing the activities of glucose-6-phosphate and phosphoenolpyruvate carboxy-kinase (Hayanga et al., 2016). The combination of various bioactive compounds in grapes can contribute to regulating glucose metabolism and improve metabolic conditions.

Furthermore, the natural antioxidant compounds in grapes, such as phenols and flavonoids, play an important role in oxidative stress-related disorders, including diabetes. Oxidative stress can be effectively neutralized by enhancing cellular defenses through antioxidants. Antioxidants can inhibit free radicals through the mechanism of redox reactions. Oxidation reactions in the body can produce free radicals, which initiate a chain reaction that damages cells. Antioxidants can end this chain reaction by removing free radical intermediates and inhibiting other oxidation reactions by oxidizing themselves (Ardani et al., 2023). It has been proven that antioxidant compounds have pharmacological action as an antidiabetic agent. Grapes have bioactive compounds that can be used as an alternative to regulate blood glucose levels and reduce oxidative stress levels linked with type 2 diabetes (Naz et al., 2023).

After treatment, blood glucose levels in the positive control group decreased from 743.4 mg/dL to 294.4 mg/dL, averaging 441.7 mg/dL. Glibenclamide was employed as the positive control. Glibenclamide is an oral antidiabetic medication shown to lower blood glucose levels. Glibenclamide is a second-generation sulfonylurea commonly used by diabetic patients (Widyastuti et al., 2022). NaCMC at 0.5% is used to dissolve glibenclamide due to the insolubility of glibenclamide in water. In experimental animals, na-CMC does not impact blood glucose levels because rats lack the enzyme cellulase in their digestive systems (Indrawati et al., 2015).

The normal group consists of non-DM rats provided with standard food and water during the treatment. The normal group's blood glucose levels increased from 67.3 mg/dL to 107.3 mg/dL. The increase in blood glucose levels is caused by several factors, such as the rats' physiological condition and stress during treatment. These conditions potentially disrupt the hormone activity that regulates blood glucose levels, which can lead to an increase in blood glucose levels through the release of cortisol and adrenaline hormones (Saputra et al., 2018).

Furthermore, the blood glucose level reduction data was statistically analyzed with an Independent T-test test. The test results showed a p-value of 0.533 ($p>0.05$), proving no significant difference between the green grapefruit extract group and the positive control group. This similarity between the two groups provides the potential of green grapefruit extract as an antidiabetic agent. Based on the test results, it can be said that in this study, green grapefruit extract can reduce blood glucose levels significantly and positively control in diabetic rat models.

The efficacy of green grapefruit extract in reducing blood glucose levels was calculated using Equation 1. The percentage of efficacy in reducing blood glucose levels of the diabetic animal model is presented in Figure 3. The results revealed a remarkable efficacy of 98.4% in reducing blood glucose levels of diabetic model animals compared to the positive control. This high efficacy proves the potential of green grapefruit extract to lower glucose levels and manage diabetes in experimental animals effectively.

This study has several limitations, including taking blood from experimental animals, which requires special skills, so it must be done with expert supervision. Secondly, the large number of test animals caused the blood sampling process to take

longer. In addition, experimental animals died during the research process, which reduced the number of samples and potentially affected the results. This study also did not determine the standard dose given. To overcome these limitations, further research is suggested to establish a standardized optimal dosage. Besides that, it is important to conduct detailed investigations into the molecular pathways affected by grapefruit extracts, assess long-term effects, and implement clinical trials to evaluate efficacy and safety in human subjects. Despite these limitations, the potential of green grapefruit extracts as effective antidiabetic agents remains significant, warranting further exploration and development in clinical applications.

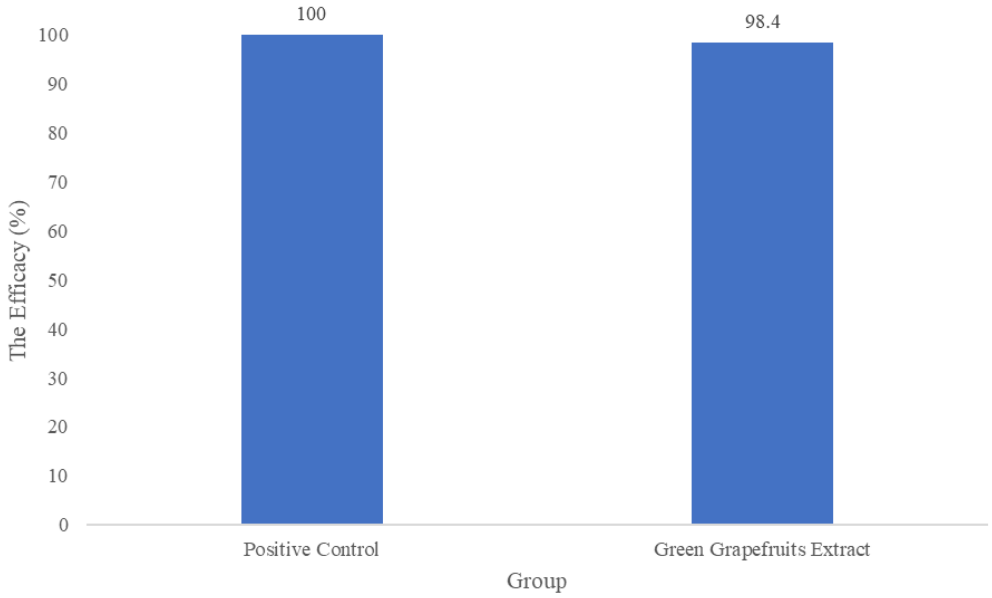

Figure 3. The Percentage of Efficacy in Reducing Blood Glucose Levels

CONCLUSION

Based on the results obtained, it can be concluded that green grapefruit extract was effective in decreasing blood glucose levels in diabetic animal models compared to the positive control with a percentage efficacy of 98.4%. These results proved the ability of green grapefruit extract to lower blood sugar and manage diabetes in the experimental animals. Further research is suggested to establish a standardized optimal dose. In addition, it is necessary to study the mechanism pathway of green grapefruit extract as an antidiabetic agent, assess long-term effects, and implement clinical trials to evaluate efficacy and safety in human subjects.

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CONFLICT OF INTEREST

There is no conflict of interest related to this research and publication.

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