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The Potential of Rosella (*Hibiscus sabdariffa* L.) for Diabetes Mellitus Model Rats: Histopathological Focus on Pancreatic Organ

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ABSTRACT

Introduction. Ethanolic extract of rosella flowers (*Hibiscus sabdariffa* L.) contains secondary metabolite compounds: flavonoids, alkaloids, saponins, and tannins. This study aims to determine whether the ethanol extract of rosella flowers has the potential to regenerate pancreatic β cells in white rats (*Rattus norvegicus*) with alloxan-induced diabetes mellitus models. **Methods.** The type of research used was an in vivo laboratory experimental study with a true experimental design. The experimental animals used as test objects in this study were male rats (*Rattus norvegicus* L.), weighing 100-110 grams. The test objects were 30 rats, divided into 6 groups randomly, each containing 5 rats, the groups consisted of K (N), K (-), K (+), P (1), P (2), and P (3). The level of histopathological damage to the pancreas was observed with HE staining at 400x magnification, using binocular light microscope type CX23 and BX51. **Results**. The study showed that the ethanol preparation of rosella flower extract at a dose of 1500 mg/kgBW and 3000 mg/kgBW BB was effective in regenerating pancreatic β cells with a damage value of 0 and at a dose of 750 mg/kgBW with a damage value of 2 did not provide a maximum regeneration effect on pancreatic β cells in male white rats induced by alloxan. **Conclusion.** The results of this research show that the ethanol extract of rosella flowers has an influence on the regeneration of pancreatic β cells so that it can be used for supportive treatment in the management of diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels, leading to complications in the heart, blood vessels, eyes, kidneys, and nerves. Type 2 DM, the most prevalent form, results from insulin resistance or insufficient insulin production. Persistent hyperglycemia (>150 mg/dL) contributes to pancreatic damage, particularly affecting the islets of Langerhans, leading to structural and functional impairments.[1]

DM can be prevented by implementing a healthy diet, undergoing regular exercise, maintaining ideal body weight, managing stress well, and checking blood sugar regularly.[2] While antidiabetic drugs help control blood glucose, they often cause side effects, prompting research into safer alternatives like herbal medicine. The petals of rosella flowers (*Hibiscus sabdariffa L.)* are one of the herbal plants that are effective as a source of natural antioxidants. Many people utilize this plant as food and drink. Antibacterial, antifungal, antiparasitic, antipyretic, antinociceptive, anti-inflammatory, nephroprotective, diuretic, anticancer, hepatoprotective, anticholesterol, antiobesity, antidiabetic, antihypertensive, and antianemia are just a few of the numerous pharmacological properties of *Hibiscus sabdariffa L.*[3]

Excessive exposure to free radicals is the cause of many chronic diseases nowadays, including diabetes mellitus. Rosella (*Hibiscus sabdariffa L.*) is also known for its bioactive compounds including gossypetin, anthocyanins, and hibiscine glycosides. Free radicals can be avoided because of the rosella flowers' strong antioxidant content.[4] Studies have shown that rosella extract can significantly reduce blood glucose levels in diabetic rats, with doses of 500 and 750 mg/kg BW yielding effects comparable to glibenclamide.[5] Additionally, a 750 mg/kg BW dose has been identified as optimal for β-cell regeneration in

streptozotocin-induced diabetic rats.[6] However, existing research is limited to specific doses and animal models. More studies are needed to explore higher doses, long-term effects, and potential clinical applications of rosella extract for pancreatic repair in DM. This study aims to determine whether the ethanol extract of rosella flowers has the potential to regenerate pancreatic β cells in white rats (*Rattus norvegicus*) with alloxan-induced diabetes mellitus models.

2. Methods

This study was an in vivo experiment with a true experimental design, conducted at the Biomedical Laboratory and Animal House, Faculty of Medicine, University of Muhammadiyah Palembang. The samples in this study were rosella flowers (*Hibiscus sabdariffa L.*) taken from plants in Pulau Panggung Village, Semende Darat Laut District, Muara Enim Regency, South Sumatra Province. Which are not contaminated by pests or fungi and are not damaged or rotten. The experimental animals used as test objects in this study were male rats (*Rattus norvegicus* L.), weighing 100-110 grams.

The test objects were 30 rats, divided into 6 groups randomly, each containing 5 rats, the groups consisted of K (N), K (-), K (+), P(1), P(2), and P(3). The selection of test subjects, grouping, and treatment administration followed a Completely Randomized Design (CRD) with six groups. In this experiment, rats were divided into six groups to assess the effects of ethanol extract from rosella flowers (*Hibiscus sabdariffa L.*) on alloxan-induced diabetes.

The first group, K (N), served as a normal control, receiving only water and standard feed without alloxan induction. The second group, K (-), was given food and water but was induced with alloxan at a dose of 150 mg/kg body weight without receiving any treatment. Meanwhile, the third group, K (+), also underwent alloxan induction but was treated with glibenclamide at a dose of 0.09 mg for 14 days orally, serving as the positive control. Three treatment groups (P1, P(2), and P(3)) received both food and water along with alloxan induction. They were then administered ethanol extract of rosella flowers at varying doses. P1 received 750 mg/kg body weight of the extract, P(2) was given 1500 mg/kg body weight, and P(3) received the highest dose of 3000 mg/kg body weight. Each treatment lasted for 14 days and was administered orally.

In previous research, it was also stated that preparation of Rosella flowers (*Hibiscus sabdariffa L.*) at a dose of 750 mg/kg body weight was the most effective dose compared to a dose of 250 mg/kg body weight and a dose of 500 mg/kg body weight in regenerating the pancreatic β cells of male rats (*Rattus norvegicus*) induced by streptozotocin.[6] Then for the toxic dose of rosella flowers, based on research by Sari et al., 2016, it was found that at a dose of 5000mg/kgBW, mice experienced silence, then restlessness, twitching, and weakness, and liver cells experienced autolysis. Therefore, it was decided that the dose used should not be more than 5000mg/kgBW.[7]

HE staining at 400x magnification, was performed using Olympus brand binocular light microscope type CX23 and BX51. The test data were analyzed through Mann-Whitney and for observations that showed significant differences ($\alpha < 0.05$). This research has been declared to have passed ethical review from the humanities and Islamic Medicine Bioethics Committee, Faculty of Medicine, Muhammadiyah University of Palembang with number 058/EC/KBHKI/FK-UMO/XI/2024.

Extraction and Maceration of Rosella

The process of creating dried rosella flower simplicia involves removing the seeds from the petals, washing them under running water to get rid of any debris, and then discarding the water. The petals of rosella flowers are then allowed to dry indoors for a few days without exposure to sunshine. Additionally, basic dried Rosella flowers were first blended into a powder of 500 grams of simplicia powder were placed in a maceration vessel, and 96% ethanol solvent was added until the powder was completely submerged, allowed to stand, and then periodically re-entered. Maceration should be done five times in 24 hours until the outcome approaches concentrated color. The results of maceration are filtered and collected. Evaporation dissolution is carried out using a rotary evaporator until the rosella extract is obtained, then thicken it in a water bath until the rosella flower extract is obtained.

Phytochemical Test

To analyze the presence of bioactive compounds in the ethanol extract of rosella (*Hibiscus sabdariffa L.*), several phytochemical tests were conducted. For alkaloid identification, 0.5 g of ethanol extract was dissolved in 10 mL of 2N HCl. The sample was then divided into two test tubes. In the first tube, the addition of two drops of Mayer's reagent resulted in the formation of a white precipitate, indicating the presence of alkaloids. In the second tube, the addition of Wagner's reagent produced a reddish-brown precipitate, further confirming the presence of alkaloids.[8]

To detect flavonoids, 0.5 g of the ethanol extract was dissolved in 5 mL of 70% ethanol. A few drops of 2N concentrated HCl and 0.2 g of magnesium powder were then added. The formation of a dark red or reddish-black precipitate confirmed the presence of flavonoids in the sample.[8]

Saponins were identified by dissolving 0.5 g of ethanol extract in 10 mL of hot water, allowing the solution to cool, and then shaking it vigorously. After adding two drops of HCl, the sample was observed for the presence of persistent foam. If the foam remained stable and did not disappear within a few seconds, the

sample was considered positive for saponins.[8]

To detect tannins, 0.5 g of the extract was dissolved in 5 mL of hot water and thoroughly shaken. The addition of 2–3 drops of 1% $FeCl_3$ solution resulted in a dark blue or black coloration, indicating the presence of tannins in the sample.[8]

Preparation of Alloxan Induction Solution

To induce diabetes in rats, alloxan was administered at a dose of 150 mg/kg body weight. For a typical rat weighing around 100 grams, the required dose was dissolved in 1 mL of NaCl and administered using a 1 cc syringe.[9]

Preparation of Glibenclamide Suspension

For the preparation of the glibenclamide suspension, the standard human dose of 5 mg was converted to an equivalent dose for rats using a conversion factor. Based on this adjustment, the appropriate dose for the rats was calculated to be 0.09 mg. This dose was then administered in a total volume of 2 mL to ensure accurate and even distribution.[10]

To prepare a 50 mL stock solution, the amount of glibenclamide required was calculated to be 2.25 mg. This amount was carefully weighed and dissolved to create a uniform suspension. If glibenclamide tablets were used instead of pure powder, the total weight of the tablets needed was also determined. Given that each tablet weighs 201.8 mg and contains only 5 mg of active glibenclamide, the required amount of powdered tablet was measured to be 90.8 mg. This ensured that the correct amount of the active compound was used while accounting for the additional ingredients present in the tablet formulation.[10]

The supply solution was prepared by sprinkling 0.5 grams of sodium carboxymethyl cellulose (Na CMC) in a mortar with 10 ml of hot distilled water. The mixture was left to stand for 15 minutes until a transparent mass was formed, and then it was agitated until it was homogenous. A 100 ml volumetric flask was filled with the Na CMC suspension. Distilled water was added to the volume until it reached 50 ml.[10]

3. Results

3.1. Phytochemical Test of Ethanol Extract of Rosella Flower

The results of the phytochemical compound test of Rosella flowers show the presence of alkaloids, flavonoids, tannins, and saponins (Table 1).

3.2. Rat Blood Glucose Level

Based on table 2, it can be seen that there is an increase in blood glucose in rats in groups K(-), K(+), P(1), P(2), and P(3) on pre-induction, the 3rd day after the administration of alloxan at 150 mg/kgBW. In contrast, the rats in the normal group (K(N) showed no such increase. On the 14th day after the intervention using rosella extract, the P(1) group obtained a change of 8%, P(2) by 74%, and P(3) by 46%. While in group K(+) that received glibenclamide intervention, a change of 42% was obtained.

This study shows that there were no changes in the blood glucose levels of rats in the K (N) and K (-) groups because the termination was immediately carried out in both groups of rats on the 3rd day after alloxan induction for ethical reasons. However, it can be seen in the K (-) group rats, that there was a notable increase in blood glucose after alloxan induction, with a rise of 160%. The decrease in blood glucose levels with rosella extract intervention was observed in groups P (1), P (2), and P (3) with the highest decrease occurring in group P (2) with a dose of 1500mg/kgBW.

3.3. Effect of Rosella Extract on Rat Pancreas Histology

As shown in Figure 1(A), the islets of Langerhans appear normal, with well-defined boundaries, a normal number and shape of Langerhans cells, and no signs of necrosis. Therefore, the histopathological score for K(N) is 0, indicating normal conditions. In Figure 1(B), the islets of Langerhans exhibit morphological changes, a reduced number of Langerhans cells, and the presence of abnormal and necrotic cells. Consequently, the histopathological score for K(-) is 4. Figure 1(C) shows normal islets of Langerhans with well-preserved boundaries, a normal number and shape of Langerhans cells, and no necrotic cells, resulting in a histopathological score of 0 for K(+), indicating normal conditions. In Figure 1(D), the islets of Langerhans display early morphological changes, with less distinct cell boundaries and a reduced number of cells, corresponding to a histopathological score of 2 for P(1). Finally, Figures 1(E) and 1(F) show normal islets of Langerhans, with well-defined boundaries, a normal quantity and shape of Langerhans cells, and no signs of necrosis, leading to histopathological scores of 0 for both P(2) and P(3), indicating normal conditions.

Table 1. Phytochemical test result of ethanol extract of rosella flower

Phytochemical Test	Reagen	Result		Description
		Positive	Negative	
Alkaloid	Mayer and Wagner	√		White precipitate
Flavonoid	HCl 2N	√		Brownish red precipitate
Tannin	$FeCl_3$ 1%	√		Brownish red solution with black precipitate
Saponin	HCl	√		Stable froth

Table 2. Rat blood glucose level

Group	Mean Blood Glucose Level (mg/dl) ± SD				% of Change
	Day-0	Pre induction	Day-7	Day-14	
K (N)	128,8±13,6	128,8±13,6	128,8±13,6	128,8±13,6	0%
K (-)	82± 8,4	213,25±25,2	213,25±25,2	213,25±25,2	160%
K (+)	81±17,8	200,25±10,7	109,25±15,5	116,5±17,2	-42%
P (1)	83,2±14,1	328,4±182,1	230,8±186,5	303±221,7	-8%
P (2)	85±15,8	448,5±174,2	119±14,1	115±16,9	-74%
P (3)	91,8±7,95	337,5±108	175,75±98,8	183±143,2	-46%

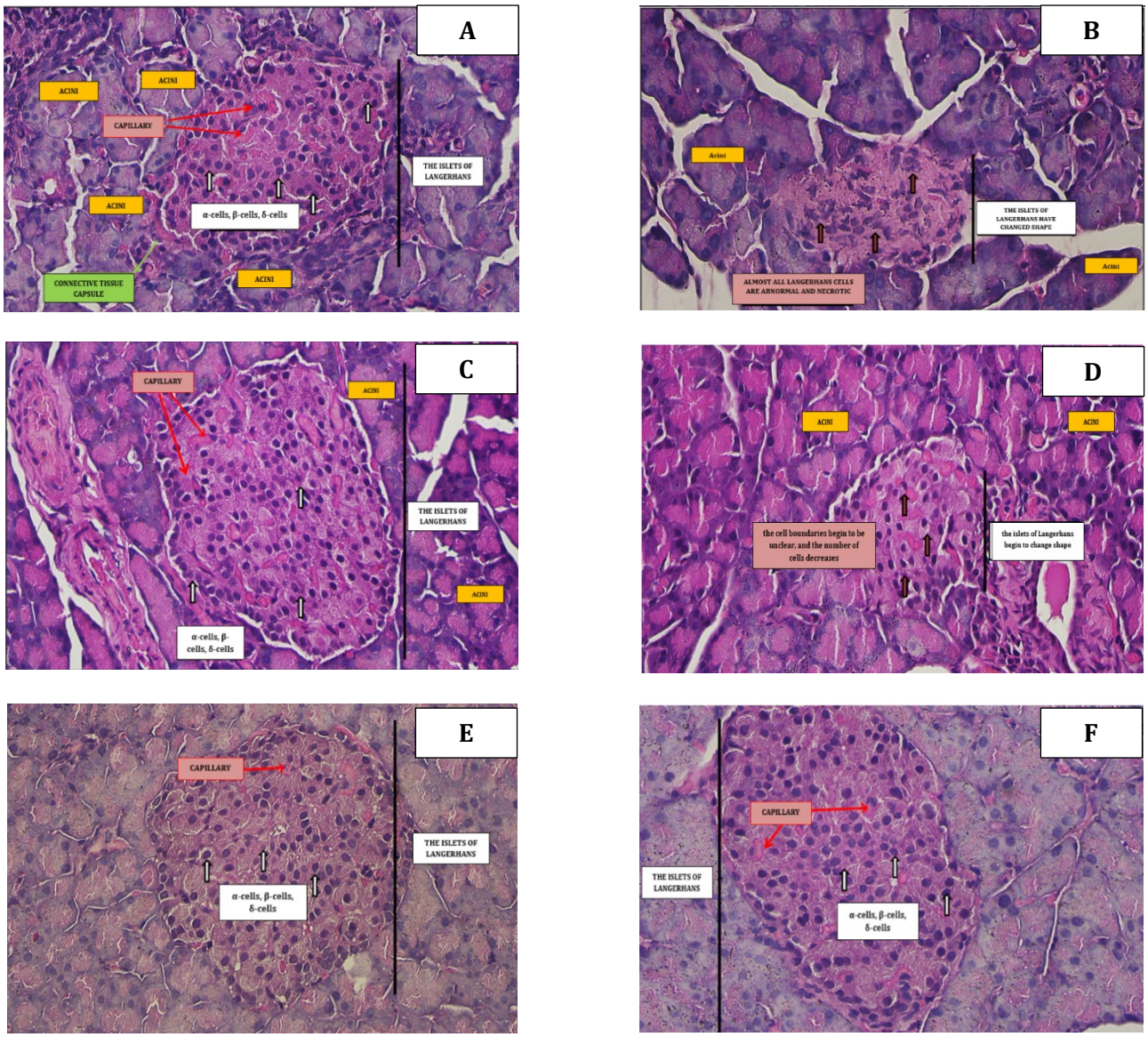

Figure 1. Histopathology of male white rat pancreas with HE staining magnification 400x.
Notes: A. K(N), B. K(-), C. K(+), D. P(1), E. P(2), F. P(3)

As shown in Table 3, significant differences were observed between the K(-) group and all other groups ($p < 0.05$). The P(1) group also differed significantly from all other groups ($p < 0.05$). In contrast, P(2) and P(3) showed significant differences only with K(-) and P(1), with no significant differences compared to K(N), K(+), or each other.

Table 3. Data analysis

Variable	Group Comparison	Mann Whitney (p value)
Normal (K(N))	Positive K(+)	1.000
	Negative K(-)	.003
	Dose 750mg/kgBW (P1)	.003
	Dose 1500 mg/kgBW (P(2))	1.000
	Dose 3000 mg/kgBW (P(3))	1.000
Negative (K(-))	Normal	.003
	Positive K(+)	.003
	Dose 750mg/kgBW (P1)	.003
	Dose 1500 mg/kgBW (P(2))	.003
	Dose 3000 mg/kgBW (P(3))	.003
Positive (K(+))	Normal	1.000
	Negative K(-)	.003
	Dose 750mg/kgBW (P1)	.003
	Dose 1500 mg/kgBW (P(2))	1.000
	Dose 3000 mg/kgBW (P(3))	1.000
Dose 750mg/kgBW (P(1))	Normal	.003
	Negative K(-)	.003
	Positive K(+)	.003
	Dose 1500 mg/kgBW (P(2))	.003
	Dose 3000 mg/kgBW (P(3))	.003
Dose 1500 mg/kgBW (P(2))	Normal	1.000
	Negative K(-)	.003
	Positive K(+)	1.000
	Dose 750mg/kgBW (P1)	.003
	Dose 3000 mg/kgBW (P(3))	1.000
Dose 3000 mg/kgBW (P(3))	Normal	1.000
	Negative K(-)	.003
	Positive K(+)	1.000
	Dose 750mg/kgBW (P1)	.003
	Dose 1500 mg/kgBW (P(2))	1.000
	Dose 3000 mg/kgBW (P(2))	1.000

4. Discussion

4.1. Phytochemical Test of Ethanol Extract of Rosella Flower

In this study, several phytochemical tests were carried out on rosella extract, it was found that rosella extract positively contained several types of antioxidants, namely alkaloids, flavonoids, saponins, and tannins. This is in line with research from Adinda et al, 2023, that in the phytochemical test of rosella extract, positive results were obtained in alkaloid compounds, phenolics, glycosides, cardioglycosides, flavonoids, quinones, saponins, tannins, coumarins, terpenoids, and anthocyanins. Negative results on betasianin and steroids.[11]

4.2. Rat Blood Glucose level

This study shows that rats in negative control, positive control, 750mg/kgBW, 1500mg/kgBW and 3000 mg/kgBW rosella extract groups had higher blood glucose levels following alloxan induction. This demonstrates the damaging effect of alloxan on pancreatic beta cells, leading to reduced insulin production and elevated blood glucose levels. Alloxan, a urea-derived compound, is selectively toxic to pancreatic beta cells due to its accumulation via the glucose transporter GLUT2. Once inside the cells, alloxan undergoes reduction, forming dialuric acid, which initiates a redox cycle that generates superoxide radicals. These radicals are then converted into hydrogen peroxide by superoxide dismutase, resulting in increased reactive oxygen species (ROS). The rise in ROS levels triggers a surge in cytosolic calcium concentration, ultimately causing rapid beta cell death and further contributing to hyperglycemia.[2]

The precise mechanism by which glibenclamide lowers blood glucose levels may be the reason for the drop in blood glucose levels observed in rats in the positive control group following glibenclamide intervention. Glibenclamide works by blocking potassium channels in pancreatic beta cells that are sensitive to adenosine triphosphate (ATP). This depolarizes the membrane, which causes tension and calcium channel opening. This causes beta cells' calcium levels to rise, which in turn triggers the release of insulin.[12]

This study revealed that rosella extract effectively lowers blood glucose levels across all treatment groups. The antioxidant chemicals flavonoids, tannins, and saponins found in rosella flowers

(*Hibiscus sabdariffa* L.) may be the reason for the decrease in blood glucose levels. By counteracting the rise in reactive oxygen species (ROS) brought on by diabetes, flavonoids can prevent the production of free radicals, allowing the pancreatic β cells to repair damage and overcome insulin insufficiency.[6] Additionally, flavonoids play a role in restoring insulin receptor sensitivity and enhancing insulin sensitivity, contributing to improved glucose regulation.[14] Other substances, like tannins, can impact diabetes conditions by suppressing nutrition by preventing the intestinal absorption of glucose and promoting the regeneration of pancreatic β-cells, which in turn affects adipose cells and raises insulin activity.[16]

The antioxidant activity of rosella petal extract enhances the capacity of intracellular antioxidant enzymes by reducing the need for their activation. In glucose-utilizing cells, this mechanism helps mitigate the harmful effects of reactive oxygen species and reduces hyperglycemia associated with type 2 diabetes. Therefore, rosella petal extract exhibits both antioxidant and antihyperglycemic properties[2] This finding aligns with research by Dianasari and Aprila (2015), which suggests that the active compounds in rosella flowers responsible for lowering blood glucose levels include flavonoids — particularly anthocyanins — as well as vitamin C and polysaccharides (pectin and mucilage). Vitamin C and flavonoids, especially anthocyanins, act as potent antioxidants, protecting pancreatic beta cells from free radical-induced damage and reducing the risk of diabetes-related complications.[4]

Interestingly, the 1500 mg/kg BW rosella extract group showed the most significant decrease in glucose levels, despite not receiving the highest dose. This finding contrasts with previous studies, such as Dianasari and Aprila (2015), which suggested a dose-dependent antidiabetic effect. The discrepancy may be attributed to variations in the source and quality of the rosella plants used.[4] This research study aligns with the research by Sanou et al. (2022), which highlights the role of environmental factors—such as temperature, precipitation, and relative humidity—in shaping the phytochemical and nutritional profiles of plants. These factors directly influence the concentration of bioactive compounds, particularly phenolics and flavonoids, which play a key role in the antidiabetic activity of rosella extract. Additionally, genetic traits, modulated by growing conditions and seasonal variations, can alter metabolite composition and potency, contributing to variability in therapeutic effects. The antidiabetic effects of rosella extract are primarily attributed to its high antioxidant content, particularly phenolic and flavonoid compounds. These antioxidants help reduce oxidative stress in pancreatic β-cells, enhance insulin secretion, and contribute to better blood glucose regulation.[13]

However, the variance observed between doses 1500 mg/kgBW vs 3000 mg/kgBW rosella extract suggests a possible saturation effect, where increasing the dose beyond an optimal range does not enhance efficacy. This highlights the importance of dose optimization in therapeutic applications. Groups 1500 mg/kgBW vs 3000 mg/kgBW rosella extract exhibited glucose-lowering effects comparable to the positive control treated with glibenclamide. This suggests that high doses of rosella extract may serve as a natural alternative to synthetic antidiabetic drugs. While glibenclamide reduces blood glucose by stimulating insulin secretion through ATP-sensitive potassium channel inhibition, rosella extract likely exerts similar effects through its antioxidant and anti-inflammatory properties.[12] This study highlights the potential of rosella extract as a plant-based intervention for diabetes.[17,18] However, it also underscores critical factors influencing its efficacy, such as source quality, environmental conditions, and dose optimization.[19] These findings emphasize the need for standardized cultivation practices and rigorous testing to ensure consistency in therapeutic outcomes. Additionally, exploring synergistic effects with other antidiabetic agents or compounds could further enhance its effectiveness.[20]

4.2 Effect of Rosella Extract on Rat Pancreas Histology

The findings of this study provide a comprehensive evaluation of pancreatic β-cell integrity across six experimental groups using histopathological scoring, revealing key insights into the protective and therapeutic potential of various treatments against alloxan-induced damage. The damage in the negative control group shows that the islets of Langerhans are malformed, there are fewer Langerhans cells, and nearly all of the cells are aberrant and necrotic. This demonstrates that giving rats alloxan can harm their pancreatic beta cells, which lowers insulin production and raises blood glucose levels. Because alloxan accumulates and may specifically pass through the glucose transporter, it is selectively harmful to the beta cells of the pancreas that make insulin, which can raise blood glucose levels. GLUT 2.[2] This is in line with research from Novitashari et all[15], which explained that alloxan injection would be able to create hyperglycemia conditions in experimental animals.

The study demonstrates that the glibenclamide-treated group maintains a normal quantity and morphology of Langerhans cells, with no signs of necrosis, and well-preserved islet boundaries. This shows glibenclamide treatment effectively preserves pancreatic β-cell morphology, yielding a histopathological score of 0, comparable to the normal control. Glibenclamide exerts its therapeutic effect by inhibiting ATP-sensitive potassium channels in β-cells, leading to membrane depolarization, calcium influx, and enhanced insulin secretion. This result emphasizes glibenclamide's efficacy in mitigating the deleterious effects of alloxan and

preserving pancreatic β-cell function.[12]

The study shows 750 mg/kg BW rosella extract gives partial restoration compared to the negative control group. However, these changes remain suboptimal relative to the normal control group and glibenclamide-treated group. This suggests a dose-dependent effect, where rosella extract at this concentration provides partial protection against oxidative stress but does not fully restore β-cell integrity. In contrast, treatment with 1500 and 3000 mg/kg BW rosella extract achieved complete restoration, with pancreatic islets exhibiting normal morphology, intact boundaries, and preserved cell density, comparable to the normal control group and glibenclamide-treated group. This suggests that rosella not only controls blood sugar but also repairs pancreatic damage, which is crucial for long-term DM management. The presence of flavonoids, tannins, saponins, and alkaloids helps reduce oxidative stress and inflammation, which are key drivers of β-cell destruction in DM. Flavonoids, in particular, protect against reactive oxygen species (ROS), allowing β-cells to recover and enhance insulin production. Based on this it can be seen that Rosella extract has the potential as diabetes adjunct therapy.[14]

The absence of significant differences ($P > 0.05$) between 1500, 3000 mg/kgBW rosella extract, normal control group, and glibenclamide-treated group suggests that higher doses of rosella extract offer equivalent protective effects to glibenclamide, highlighting its potential as a viable natural therapeutic agent against β-cell damage. Overall, these findings confirm that alloxan significantly impairs pancreatic β-cells, inducing diabetes-like conditions in the negative control group, while glibenclamide and high-dose rosella extract (1500 and 3000 mg/kg BW rosella extract) effectively prevent damage and restore pancreatic function, suggesting the potential of rosella extract, especially at higher doses, as a promising natural treatment for β-cell protection.[2]

While this study demonstrates that rosella extract can regenerate pancreatic β-cells, the specific molecular pathways behind this effect remain unclear. The observed improvements in pancreatic histology and blood glucose levels suggest that antioxidant and anti-inflammatory mechanisms play a key role. However, without detailed biochemical analysis, it is difficult to determine how rosella promotes β-cell repair and function. To address this, future research should explore, Gene Expression Analysis by investigating β-cell regeneration markers such as Pdx-1, Ngn3, and Insulin genes could confirm whether rosella stimulates β-cell neogenesis or enhances insulin production. Find out the inflammatory pathways by examining the expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) could reveal whether rosella reduces inflammation-driven β-cell destruction, which is a crucial step in long-term pancreatic recovery. Examining the insulin secretion assays and conducting insulin release tests would help determine whether the regenerated β-cells regain functional insulin secretion, confirming rosella's potential beyond structural repair. By addressing these gaps, future research can establish whether rosella not only regenerates pancreatic β-cells but also restores their full function, making it a promising candidate for natural diabetes therapy.

5. Conclusion

The ethanol extract of rosella (*Hibiscus sabdariffa L.*) at various doses demonstrates potential in regenerating pancreatic β-cells in alloxan-induced diabetic rats. Among the tested doses, 1500 mg/kg body weight proved to be the most effective in regenerating β-cells. This dose resulted in the highest reduction in blood glucose levels, showing a 74% decrease, and achieved a pancreatic histopathology score of 0, signifying full restoration. This suggests that the 1500 mg/kg dose had a greater therapeutic effect compared to the lower dose of 750 mg/kg and the higher dose of 3000 mg/kg. Its antioxidant and anti-inflammatory properties, primarily due to bioactive compounds like flavonoids, tannins, and saponins, contribute to these effects. Rosella extract has demonstrated significant potential in reducing blood glucose levels and regenerating pancreatic β-cells, making it a promising natural therapy for diabetes. However, the study findings highlight the importance of dose optimization and the influence of environmental and genetic factors on its efficacy. Future research should focus on molecular mechanisms, anti-inflammatory pathways, and functional insulin secretion tests to better understand how rosella extract promotes β-cell repair and function. Standardized formulations and clinical trials could further establish rosella as an effective plant-based intervention for diabetes management.

6. Author Contribution

SJLP contribution to data collection, and writing original manuscript under the supervision of SR. SR designed the research, methodology, conceptualization of the research, investigation process, supervision of the research, validation, review, and editing. ES contribution in methodology, analyzed, and validation the research data. YA carried out the methodology, analyzed and validation research data. TM contribution the conceptualization of the research. SR and ES have approved the final manuscript.

7. Acknowledgements

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