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A Review of the Impact of Stevia Leaf (*Stevia rebaudiana* Bert.) as a Sweetener in Blood Glucose in Alloxan-Induced Rats

Refitha Nurul Putri[1*], Patihul Husni[2]

[1]Pharmacist Professional Study Program, Faculty of Pharmacy, Universitas Padjadjaran

[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran

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*Corresponding author: refitha161202@gmail.com

Abstract

Diabetes mellitus (DM) is a chronic condition characterized by elevated blood glucose levels due to insulin-related deficiencies. Medicinal plants have been widely explored as alternative therapies for managing DM. Among these, *Stevia rebaudiana* Bert, which known as a natural sweetener 200–300 times sweeter than sucrose, boasts a zero glycemic index, making it particularly suitable for individuals with diabetes. This review aims to evaluate the antihyperglycemic potential of *S. rebaudiana* Bert. by focusing on its efficacy in alloxan-induced diabetic rat models. A literature review was conducted by acquiring data from scientific databases such as Semantic Scholar, PubMed, Scopus, Crossref, and ScienceDirect, focusing on literature published between 2020–2024. Studies on diabetic rat models induced with alloxan were analyzed, with doses ranging from 20–1000 mg/kgBW and optimal doses identified between 120–150 mg/kgBW. The results demonstrated that Stevia significantly reduced blood glucose levels in diabetic rats and sustained hyperglycemia, effectively modeling type 1 diabetes. The bioactive constituents, including phenolics, alkaloids, glycosides, steviosides, flavonoids, and tannins, were identified as key contributors to its antihyperglycemic activity. In conclusion, *S. rebaudiana* Bert. exhibits promising efficacy as a natural antidiabetic agent. Its ability to reduce blood glucose levels and its zero glycemic index position it as a viable alternative to conventional antihyperglycemic therapies, especially for managing type 1 DM.

Keywords: Alloxan, Diabetes Mellitus, In Vivo, Stevia, Sweetener.

Ulasan Dampak Daun Stevia (*Stevia rebaudiana* Bert.) sebagai Pemanis terhadap Glukosa Darah pada Tikus yang Diinduksi Aloksan

Abstrak

Diabetes melitus (DM) adalah suatu kondisi kronis yang ditandai dengan peningkatan kadar glukosa darah akibat kekurangan insulin. Tanaman obat telah banyak dieksplorasi sebagai terapi alternatif untuk mengelola DM. Di antaranya, *Stevia rebaudiana* Bert., dikenal sebagai pemanis alami yang 200-300 kali lebih manis dari sukrosa dan memiliki indeks glikemik nol sehingga sangat cocok untuk penderita diabetes. Tinjauan ini bertujuan untuk mengevaluasi potensi antihiperglikemik dari *S. rebaudiana* Bert., dengan fokus pada efektivitasnya pada model tikus diabetes yang diinduksi alloksan.. Tinjauan sistematis dilakukan dengan memperoleh data dari basis data ilmiah seperti *Semantic Scholar, PubMed, Scopus, Crossref,* dan *ScienceDirect*, dengan fokus pada literatur yang diterbitkan antara tahun 2020-2024. Studi pada model tikus diabetes yang diinduksi dengan aloksan dianalisis, dengan dosis berkisar antara 20-1000 mg/kgBB dan dosis optimal diidentifikasi antara 120-150 mg/kgBB. Hasil penelitian menunjukkan bahwa Stevia secara signifikan mengurangi kadar glukosa darah pada tikus diabetes dan hiperglikemia yang berkelanjutan, yang secara efektif memodelkan diabetes tipe 1. Senyawa bioaktif termasuk fenolik, alkaloid, glikosida, steviosida, flavonoid, dan tanin diidentifikasi sebagai kontributor utama untuk aktivitas antihiperglikemik. Kesimpulannya, *S. rebaudiana* Bert. menunjukkan kemanjuran yang menjanjikan sebagai agen antidiabetes alami. Kemampuannya untuk menurunkan kadar glukosa darah dan indeks glikemik nol memposisikannya sebagai alternatif yang layak untuk terapi antihiperglikemik konvensional, terutama untuk mengelola DM tipe 1.

Kata Kunci: Aloksan, Diabetes Mellitus, In Vivo, Pemanis, Stevia.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels due to insufficient insulin production or ineffective insulin utilization. It primarily includes two types: type 1 diabetes, which occurs when the body is unable to produce insulin, and type 2 diabetes, which usually develops due to insulin resistance.[1,12] Both conditions present significant health risks, including complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy. The rising global prevalence of diabetes, particularly type 2 diabetes, underscores the necessity for effective dietary management strategies. Among these strategies, the use of alternative sweeteners plays a crucial role, as they provide the opportunity to enhance flavor without substantially impacting blood glucose levels. These sweeteners serve as an essential resource for individuals managing diabetes, including those with type 1, allowing for improved dietary satisfaction while mitigating the health risks associated with traditional sugar consumption.[1,41]

The pathophysiology of DM, particularly regarding the elevation of blood glucose levels, involves a series of intricate processes that begin in the digestive system. When food is ingested, the stomach plays a critical role in breaking down the food into its constituent nutrients. Carbohydrates are specifically converted into glucose, a vital energy source, which then enters the bloodstream, resulting in an increase in blood sugar levels. In response to this elevation, the pancreas, an essential endocrine organ located behind the stomach, produces insulin, a hormone that is crucial for managing blood glucose levels. Insulin facilitates the uptake of glucose by the body's cells, enabling them to utilize this energy effectively. However, in individuals diagnosed with Diabetes Mellitus, this regulatory process may not function optimally. This can be attributed to insulin resistance, where the body's cells do not respond adequately to insulin, or to insufficient insulin production due to pancreatic dysfunction. As a consequence, glucose remains in the bloodstream rather than being absorbed by the cells, leading to persistent hyperglycemia, or elevated blood sugar levels (Fig. 1).[1,41]

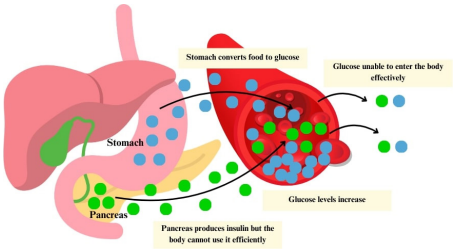

Fig. 1. Pathophysiology of Diabetes Mellitus in Increasing Blood Sugar Levels in the Pancreas/Stomach.

Stevia rebaudiana Bert., commonly known as Stevia, is a perennial herb indigenous to South America, particularly Paraguay and Brazil. It has gained prominence as a natural sweetener due to the presence of several active compounds in its leaves, primarily steviosides and rebaudiosides. These glycosides exhibit sweetness levels that are approximately 250 to 300 times greater than sucrose, making them a favorable alternative for enhancing flavor without contributing to caloric intake or adversely affecting blood glucose levels.[2] Steviosides are the principal sweet components, while rebaudiosides A and C have been extensively studied for their sweetness intensity and potential health benefits. The non-caloric properties of these compounds render stevia particularly appealing for individuals with diabetes and those seeking to reduce overall sugar consumption.[3]

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil), a synthetic analog of uracil, is known for its selective toxicity toward pancreatic beta cells, which are essential for the production and secretion of insulin. When administered to rats, typically through an injection, alloxan is rapidly distributed throughout the bloodstream and subsequently targets the pancreas. Within the islets of Langerhans, alloxan exerts its detrimental effects primarily through the generation of reactive oxygen species (ROS), which induce oxidative stress in beta cells. This oxidative stress overwhelms the cells' inherent antioxidant defenses, leading to mitochondrial dysfunction, significant DNA

damage, and ultimately, cellular apoptosis.[4] As the population of beta cells declines due to the action of alloxan, there is a marked reduction in the secretion of insulin. Insulin is a critical hormone responsible for regulating blood glucose levels by facilitating the uptake of glucose by various tissues. Therefore, the impairment of insulin production resulting from beta-cell destruction leads to elevated blood glucose levels, a characteristic feature of diabetes mellitus.[5]

The urgency of this study is driven by the increasing global prevalence of diabetes mellitus and related metabolic disorders, which are becoming major public health concerns. Current therapeutic options often come with limitations such as side effects, high costs, and insufficient long-term efficacy, highlighting the need for safe, affordable, and effective alternatives. *S. rebaudiana* Bert., known for its natural origin, low-calorie profile, and zero glycemic index, presents a promising solution as a dietary intervention for glycemic control. However, there are significant research gaps in understanding the full potential of Stevia in diabetes management. While several studies have investigated its antidiabetic properties, they often have limitations, such as small sample sizes or lack of long-term follow-up, and there is a need for further evidence supporting its efficacy and safety. Furthermore, to date, comprehensive reviews specifically discussing Stevia as a glycemic control agent are limited. Therefore, the objective of this study is to evaluate the antihyperglycemic potential of Stevia through a critical review of existing literature, identifying optimal dosages, and analyzing the role of its bioactive compounds in managing diabetes mellitus, particularly in comparison with current conventional therapies. By elucidating the biochemical pathways through which Stevia enhances insulin sensitivity and modulates glucose absorption, this study aims to provide evidence-based insights into its potential role in diabetes management. The findings are critical for supporting clinical practice by offering a natural and accessible alternative to traditional sweeteners that often adversely affect blood glucose levels and metabolic health.[6]

2. Methods

A literature review was conducted using international, peer-reviewed databases, including Semantic Scholar, PubMed, Scopus, Crossref, and ScienceDirect. Only peer-reviewed journal articles published between 2020-2024 were included; grey literature, preprints, theses, or non-peer-reviewed reports were excluded to minimize potential bias. The search strategy employed full Boolean strings combining terms such as ("*Stevia rebaudiana*" OR "stevia") AND ("diabetes mellitus") AND ("alloxan") AND ("in vivo" OR "animal model"). Titles and abstracts were screened independently by two reviewers, followed by full-text screening based on inclusion and exclusion criteria. Discrepancies were resolved through discussion or consultation with a third reviewer. Risk of bias was assessed qualitatively based on clarity of methodology, reporting of controls, sample sizes, and reproducibility indicators. Given the focused scope of this review, only in vivo studies utilizing alloxan-induced diabetic animal models and reporting measurable glycemic outcomes were included. Studies published prior to 2020, those unrelated to *S. rebaudiana* Bert., or lacking alloxan-induced in vivo models were excluded. Ultimately, this review included 10 primary experimental articles meeting all criteria and 23 secondary references for background and contextual support.

3. Result and Discussion

3.1. Stevia as a Sweetener and Its Mechanism of Action

Stevia, scientifically known as *S. rebaudiana* Bert., is a plant recognized for its naturally sweet leaves, offering a beneficial alternative to synthetic sweeteners and refined sugars. The sweetness of stevia is primarily attributed to a class of compounds known as glycosides, with stevioside and rebaudioside A being the most prevalent. These glycosides are significantly sweeter than sucrose, while contributing minimal calories, making stevia an attractive option for those aiming to reduce caloric intake without compromising sweetness. The potential health benefits associated

with stevia extend beyond its flavor profile, particularly in relation to blood glucose regulation.[7]

Emerging research indicates that stevia may possess hypoglycemic properties, which are attracting significant interest from both the scientific and medical communities. One key mechanism through which stevia appears to exert its effects is by stimulating insulin secretion from the pancreas. This effect can be particularly beneficial for individuals managing type 2 diabetes and those with type 1 diabetes, as it may facilitate the incorporation of stevia into dietary strategies while continuing to rely on insulin therapy. In addition to promoting insulin secretion, stevia may play a role in inhibiting glucose absorption within the gastrointestinal tract by modulating glucose transport mechanisms. This action could contribute to a reduction in postprandial blood glucose spikes, offering an effective approach to managing glycemic responses following meals. Moreover, it is noteworthy that stevia has the potential to induce hypoglycemia in patients with diabetes by decreasing glycogenolysis and gluconeogenesis, as well as by absorbing glucose in the duodenum. The antihyperglycemic and antioxidative properties of stevia and its glycosides have been observed in several tissues, including the kidney, liver, and pancreas. This highlights the compound's potential importance in regulating blood sugar levels for individuals with diabetes.[8]

In addition to promoting insulin secretion, stevia may inhibit glucose absorption in the gastrointestinal tract. This action operates by modulating glucose transport processes, potentially leading to reduced postprandial blood glucose spikes. By minimizing the amount of glucose that enters the bloodstream, stevia presents a practical approach to managing glycemic responses following meals.[9]

Stevia extract has exhibited significant potential in enhancing pancreatic β-cell functionality within diabetic rat models, indicating its prospective role in advancing diabetes management through natural means. This effect is largely attributed to the active compounds known as steviol glycosides. Numerous studies have demonstrated that these glycosides are instrumental in modulating calcium (Ca^{2+}) oscillations occurring within pancreatic islets. These calcium oscillations are critical for β-cell function, as they facilitate the signaling pathways necessary for effective insulin secretion. Following an increase in blood glucose, particularly after meals, these oscillations enable β-cells to respond appropriately by releasing insulin, the hormone responsible for regulating blood sugar levels. Evidence suggests that steviol glycosides enhance the frequency and amplitude of these calcium fluctuations, which ultimately improves the β-cells' ability to secrete insulin in accordance with the metabolic demands presented by elevated glucose levels.[2,10]

Moreover, the protective properties of steviol glycosides on the health of pancreatic β-cells merit attention. Chronic hyperglycemia, commonly seen in diabetic conditions, often leads to β-cell stress and damage, which can further impair insulin secretion. By enhancing β-cell function and resilience, stevia shows potential for mitigating some of the adverse effects associated with diabetes.

3.2. Potential Doses of *S. rebaudiana* Bert. and Bioactive Compounds Play a Significant Role in Regulating Blood Glucose Levels

Based on the literature search process, 10 studies were obtained discussing the blood glucose-lowering activity of *S. rebaudiana* Bert., including the test doses used, the bioactive compounds contained in the plant, the number of rats used, the blood glucose levels measured, and the alloxan doses used in the induction tests on rats. These results are presented in Table 1.

The administration of herbal plants at an average dosage ranging from 20-1000 mg/kgBW has been shown to significantly reduce blood glucose levels in rat models. This reduction can be attributed to the presence of various bioactive compounds that possess distinct mechanisms contributing to their antihyperglycemic effects.

Plant-derived phenolic compounds exert their anti-diabetic effects through a variety

of mechanisms that contribute to improved glucose metabolism and insulin function. One of the key pathways involved is the activation of AMP-activated protein kinase (AMPK), which plays a crucial role in cellular energy homeostasis. When activated, AMPK promotes glucose uptake and enhances insulin sensitivity, leading to improved metabolic status. Additionally, phenolic compounds can inhibit the activities of enzymes such as α-glucosidase and α-amylase. By blocking these enzymes, they can slow down carbohydrate digestion and absorption in the gut, which helps to prevent rapid spikes in blood glucose levels after meals.[11] Furthermore, these compounds also promote glucose uptake in peripheral tissues, facilitating better utilization of glucose by muscles and fat cells. This is particularly important for managing blood sugar levels.[12] Lastly, phenolic compounds can activate peroxisome proliferator-activated receptors (PPARs), which are involved in lipid metabolism and glucose homeostasis. PPAR activation can further enhance insulin sensitivity and contribute to improved metabolic health.[13]

Alkaloids demonstrate substantial potential in the regulation of glucose levels and the enhancement of insulin action through a variety of mechanisms. Notably, these compounds have the capacity to regenerate pancreatic β-cells, which is crucial for maintaining insulin production. Additionally, alkaloids contribute to the reduction of blood glucose levels, thus supporting overall metabolic health. These substances promote insulin secretion via several extra-pancreatic pathways.[14]

They facilitate glycogen synthesis, resulting in increased glucose storage within liver and muscle tissues, while also enhancing glucose transport within the intestine. Moreover, alkaloids inhibit gluconeogenesis by targeting specific enzymes, such as glucose 6-phosphatase and fructose 1,6-bisphosphatase. Inhibition of these enzymes, which play a key role in converting non-carbohydrate substrates into glucose, subsequently diminishes glucose formation and aids in blood sugar regulation. Furthermore, alkaloids influence glucose metabolism through the modulation of various transporters and enzymes. A noteworthy focus is glucose transporter-4 (GLUT-4), which is essential for glucose uptake in muscle and adipose tissues.[14]

When insulin is present, GLUT-4 translocates to the cell membrane, facilitating glucose entry and contributing to the reduction of plasma glucose levels. Administration of alkaloids that affect GLUT-4 activity can further enhance this glucose-lowering effect. In addition to GLUT-4, alkaloids also target glycogen synthase kinase-3 (GSK-3), a critical regulator of numerous cellular processes, including insulin action. The inhibition of GSK-3 can lead to improved insulin sensitivity and more effective glucose homeostasis. Additionally, alkaloids may promote the expression of peroxisome proliferator-activated receptors (PPAR), which are involved in fat metabolism and the enhancement of insulin sensitivity.[15,16]

Glycosides and steviosides have demonstrated potential antioxidant activity. Research indicates that steviol glycosides can increase glucose uptake in rat fibroblasts by activating the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. This pathway plays a crucial role in the translocation of the glucose transporter Glut4 to the plasma membrane, thereby mimicking the action of insulin and suggesting that steviol glycosides may serve a similar function in regulating glucose metabolism.[17] Moreover, the involvement of S961, an insulin antagonist, has been shown to completely inhibit the glucose uptake effects facilitated by steviol glycosides. This finding supports the notion that steviol glycosides may act as ligands for the same receptor that insulin engages, potentially providing a novel strategy for blood glucose management.[18]

Flavonoids represent a significant category of naturally occurring plant compounds that offer numerous health benefits, particularly in the areas of metabolic regulation and the enhancement of insulin sensitivity. A key mechanism by which flavonoids exert their beneficial effects involves the phosphorylation of crucial components in the insulin signaling pathway, specifically the insulin receptor and insulin

Tabel 1. Blood Glucose-Lowering Activity of Stevia rebaudiana Bert. with Test Doses, Bioactive Compound, Rat Used, Measured Blood Glucose Level, and Alloxan Dosage

Stevia Dosage	Bioactive Compound	Rat Used (n)	Measured Blood Glucose Level	Alloxan Dosage	Result
10% extract 60% extract 100% extract	Phenolic	Inbred ICR (36)	8-Hour Fast Blood Glucose Level (fBGL) Oral Glucose Tolerance Test (OGTT)	70 mg/ kgBW	All concentrations of stevia leaf extract (100%, 60%, and 10%) showed comparable effects to glibenclamide in reducing fasting blood glucose levels. The stevia-treated groups exhibited improvements in glucose tolerance, with effects similar to glibenclamide treatment[19]
100 mg/kgBW 300 mg/kgBW 700 mg/kgBW	Alkaloid, Glycosides	Wistar (n/a)	12-Hour Fast Blood Glucose Level (fBGL) Post-Prandial Blood Glucose Level	150 mg/ kgBW	The most effective dose of microencapsulated Stevia leaf extract in reducing blood glucose levels was found to be 100 mg/kgBW[20]
500 mg/kgBW	Steviosides, Glycosides	Albino (28)	Fast Blood Glucose Level (fBGL)	60 mg/ kgBW	Significant decrease in blood glucose levels and glycated hemoglobin (HbA1c) were observed and insulin levels increased, but the treatment did not fully normalize these biomarkers after 28 days[21]
300 mg/kgBW	Glycosides, Phenolic, Flavonoids	Wistar Sprague Dawley Albino (50)	Fast Blood Glucose Level (fBGL)	120 mg/ kgBW	Dose 300 mg/kgBW reduced glucose and HbA1c, significantly reduced liver and kidney markers, indicating protective effects on these organs[22]
100 mg/kgBW 200 mg/kgBW	Flavonoids	Wistar (30)	Fast Blood Glucose Level (fBGL)	100 mg/ kgBW	Doses 100 mg/kgBW and 200 mg/kgBW significant ($P = .05$) reduction in FBG levels, indicating antidiabetic activity of *S. rebaudiana* extract[23]
60 mg/kgBW	Phenolic, Flavonoids	Male White Laboratory Rats (27)	12-Hour Fast Blood Glucose Level (fBGL)	100 mg/ kgBW	Dose 60 mg/kgBW significant reduction in blood glucose levels compared to Diabetic Control (T2)[24]
20 mg/kgBW 30 mg/kgBW	Glycosides	Wistar albino (42)	Fast Blood Glucose Level (fBGL)	150 mg/ kgBW	Doses 20 mg/kgBW and 30 mg/kgBW significantly ($P \leq 0.05$) reduced blood glucose levels compared to the alloxan-induced diabetic control (DC) group, suggesting their antidiabetic activity[25]
200 mg/kgBW 400 mg/kgBW	Tannin, Phenolic	Wistar (40)	Fast Blood Glucose Level (fBGL)	150 mg/ kgBW	Stevia treatment resulted in a 52% increase in fasting blood glucose in diabetic rats (DR), and there was a 40% increase in insulin levels in DR treated with Stevia[26]
250 mg/kgBW 500 mg/kgBW 1000 mg/kgBW	Alkaloid, Glycosides, Flavonoids, Phenolic	Holtzman albino (30)	12-Hour Fast Blood Glucose Level (fBGL)	150 mg/ kgBW	GlucoMedix® reduced blood glucose levels in a dose-dependent manner within 28 days, showing potential for managing hyperglycemia[27]
100 mg/kgBW 200 mg/kgBW 400 mg/kgBW	Phenolic	Male White Laboratory Rats (30)	Fast Blood Glucose Level (fBGL)	150 mg/ kgBW	A significant decline in glucose levels was observed in treated diabetic animals compared to the control group ($P < 0.001$)[28]

receptor substrate (IRS). This phosphorylation, which entails the addition of a phosphate group to proteins, is a vital modification that activates their physiological functions. By promoting this phosphorylation process, flavonoids enhance the sensitivity of the insulin receptor to insulin, initiating a cascade of intracellular signaling events that are essential for metabolic control.[29] Upon activation, the insulin receptor engages the phosphoinositide 3-kinase (PI3K)/Akt pathway, a critical signaling network that governs numerous cellular processes pertinent to metabolism. The activation of this pathway leads to significant downstream effects, including the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane in muscle and adipose tissues. The translocation of GLUT4 is crucial for promoting glucose uptake from the bloodstream into cells, thereby effectively reducing blood glucose levels following meals.[30] In addition to enhancing glucose uptake, flavonoids also exert a substantial influence on hepatic function. They have been shown to modulate the expression of two key enzymes: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P). PEPCK plays a critical role in gluconeogenesis, the metabolic process through which glucose is synthesized in the liver from non-carbohydrate sources. By downregulating the expression of PEPCK and G6P, flavonoids effectively suppress gluconeogenesis, contributing to a decrease in hepatic glucose production and thus supporting blood glucose homeostasis.[31]

Flavonoids also play a critical role in modulating inflammatory responses and in reducing levels of free fatty acids (FFAs) within the circulation. Elevated levels of FFAs are associated with the onset of insulin resistance, characterized by diminished responsiveness of cells to insulin. Flavonoids address this issue by lowering FFA concentrations and reducing inflammatory markers, thereby enhancing insulin action. Specifically, flavonoids mitigate the adverse effects of stress-related signaling proteins, such as c-Jun N-terminal kinase (JNK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and protein kinase C (PKC). These proteins promote inflammatory responses and can disrupt insulin signaling pathways. By attenuating their activity, flavonoids aid in restoring normal insulin signaling and enhancing overall metabolic health.[32]

Tannins represent a diverse group of polyphenolic compounds that are naturally present in a variety of plants, including *S. rebaudiana* Bert. A fundamental mechanism by which tannins aid in glucose regulation is through the inhibition of glucose-6-phosphatase, an enzyme essential for gluconeogenesis, the process by which glucose is synthesized in the liver. By inhibiting this enzyme, tannins can effectively reduce the amount of glucose released into the bloodstream from the liver, thereby playing a crucial role in preventing hyperglycemic episodes, especially in individuals who are insulin resistant or diagnosed with diabetes. Moreover, tannins function as inhibitors of the α-glucosidase enzyme, which is responsible for the breakdown of complex carbohydrates into simpler sugars in the small intestine. By inhibiting this enzyme, tannins slow down the digestion process, resulting in a delayed release of glucose into the bloodstream after meals. This effect is particularly advantageous for managing postprandial blood sugar spikes, contributing to a more gradual and controlled increase in glucose levels.[33,34]

3.3. The Optimal Dosage of Alloxan and Its Impact on Effectively Reducing Blood Glucose Levels

The optimal dosage of alloxan required to induce diabetes in rats is influenced by the specific type of diabetes being studied, due to the differing underlying mechanisms and causes associated with type 1 and type 2 diabetes mellitus. Type 1 diabetes is characterized as an autoimmune condition in which the immune system attacks the insulin-producing beta cells within the pancreas. Alloxan is a compound that selectively targets these beta cells. Upon administration of alloxan to rats, the compound is preferentially absorbed by the beta cells, attributable to their elevated expression of glucose transporters. Once absorbed, alloxan undergoes redox cycling, which leads to the

formation of reactive oxygen species (ROS). The presence of these ROS results in oxidative stress, causing significant damage to the beta cells. This damage ultimately leads to apoptosis, or programmed cell death, which markedly reduces insulin production.[35] Insulin is essential for the uptake of glucose by tissues. Therefore, a reduction in insulin levels causes an increase in blood glucose levels, which is representative of diabetes. Although the body may attempt to compensate for the resultant insulin deficiency initially, this condition ultimately results in persistent hyperglycemia.[1]

The findings of the review indicate that diabetes can be effectively induced in rat models through the administration of alloxan, with effective dosages ranging from 60-150 mg/kgBW. It is important to recognize that the specific dosage of alloxan is a crucial determinant in influencing both the severity and duration of the diabetic state in the affected animals. Among the various dosing protocols, a dosage of 150 mg/kgBW administered via intraperitoneal injection has been established as the optimal choice. This dosage is particularly favorable for several reasons. It consistently induces diabetes characterized by moderate hyperglycemia, thereby allowing for the observation of physiological changes and pathological processes without imposing severe adverse effects that may arise from higher doses. At this level, the damage to pancreatic beta cells is sufficiently pronounced to impair insulin secretion, effectively simulating the insulin-dependent nature of diabetes.[36,37]

In a previous study, it was demonstrated that the administration of alloxan at a dosage of 160 mg/kgBW effectively induces stable diabetes with minimal toxicity. This dosage facilitates the testing of new diabetes medications for a duration exceeding two months, during which the animals exhibit clear symptoms of diabetes. Such an extended timeframe is often essential for validating the efficacy of novel treatments in in vivo experiments. It is noteworthy that alloxan doses of 140 mg/kg BW and below typically result in a transient diabetic condition, with subjects returning to normal physiological values within a period of 10 days. Furthermore, prior research has indicated that doses under 150 mg/kgBW are inadequate for inducing diabetes. While some alterations in the Oral Glucose Tolerance Test (OGTT) may be observed at lower doses of alloxan, these changes are not reflected in fasting blood glucose levels. Although alloxan can induce physiological changes, it is important to recognize that animals frequently tend to recover spontaneously over time when lower doses are administered, necessitating no medical intervention.[38]

We have observed that doses of alloxan below 120 mg/kgBW in this plant potentially do not induce stable, measurable diabetes. Our findings also indicate that this plant can help lower fasting blood glucose levels. This decrease suggests improved insulin sensitivity and better glucose regulation, contributing to overall effective blood sugar control. Current blood glucose levels may also benefit from these compounds, helping to stabilize glucose throughout the day and reducing fluctuations that can lead to hyperglycemia or hypoglycemia. Postprandial blood glucose is another crucial parameter influenced by these antidiabetic compounds. We anticipate that these plants will significantly reduce postprandial glucose spikes, thereby improving glycemic control and potentially lowering the risk of complications associated with diabetes. The effectiveness of this plant is further supported by its performance in the Oral Glucose Tolerance Test (OGTT). The improved results in the OGTT suggest that *S. rebaudiana* Bert. may enhance the body's ability to manage glucose load, possibly through an enhanced insulin secretory response and improved insulin sensitivity.[39,40]

The observed antidiabetic potential of *S. rebaudiana* Bert. highlights its ability to positively influence key aspects of glucose metabolism. This plant has been shown to affect fasting, current, and postprandial glucose levels, as well as performance on the oral glucose tolerance test (OGTT). As a result, the bioactive compounds derived from Stevia are known to be effective in managing blood glucose levels. Additionally, factors such as the dosage of the plant used, rat used,

the duration of the research, and the doses of alloxan administered all contributed to the successful observation of the effects of *S. rebaudiana* Bert. in lowering blood glucose levels.

Despite the promising findings, this review has several limitations. First, the sample size across the included studies was limited which may reduce the generalizability of the results. Second, most studies employed different experimental protocols, including variations in the dosage of alloxan, duration of diabetes induction, and Stevia extract preparation making direct comparisons challenging. Third, the majority of the evidence is derived from animal models, particularly alloxan-induced diabetic rats, which may not fully replicate the pathophysiological complexity of human diabetes mellitus. Fourth, there was a lack of long-term studies assessing the sustained efficacy and safety of stevia, especially in relation to chronic glucose regulation and potential side effects. Finally, some studies did not clearly report methodological details such as randomization, blinding, and control group design, which may introduce bias and affect the reliability of the outcomes. These limitations highlight the need for more standardized, well-controlled, and large-scale studies to validate the antidiabetic effects of Stevia in both preclinical and clinical settings.

4. Conclusion

This review concludes that *S. rebaudiana* Bert. shows promising efficacy as a natural antidiabetic agent for reducing blood glucose levels, making it a viable alternative to conventional therapies. Additionally, the findings highlight the importance of optimizing Alloxan dosing to enhance the development of reliable diabetic models for future research on herbal and pharmaceutical interventions in managing diabetes.

References

1. Dilworth L, Facey A, Omoruyi F. Diabetes Mellitus and Its Metabolic Complications: The Role of Adipose Tissues. Int. J. Mol. Sci. 2021;22(14):7644
2. Peteliuk V, Rybchuk L, Bayliak M, Storey KB, Luschak O. Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. EXCLI J. 2021;20:1412-1430.
3. Schiatti-Sisó IP, Quintana SE, García-Zapateiro LA. Stevia (*Stevia rebaudiana*) as a common sugar substitute and its application in food matrices: an updated review. J Food Sci Technol. 2022;60(5):1483–1492.
4. Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. Physiol. Res. 2001;50:536-546.
5. Kliber A, Szkudelski T, Chichlowska J. Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. J Physiol Pharmacol. 1996;47:321-328.
6. Siso IPS, Quintana SE, Zapateiro LAG. Stevia (*Stevia rebaudiana*) as a common sugar substitute and its application in food matrices: an updated review. Journal of Food Science and Technology. 2022;60(2).
7. Pawar RS, Krynitsky AJ, Rader J. Sweeteners from plants-with emphasis on *Stevia rebaudiana* (Bertoni) and *Siraitia grosvenorii* (Swingle). Analytical and Bioanalytical Chemistry. 2013;405(13).
8. Assaei R, Mokarram P, Dastghaib S, et al. Hypoglycemic Effect of Aquatic Extract of Stevia in Pancreas of Diabetic Rats: PPARγ-dependent Regulation or Antioxidant Potential. Avicenna J Med Biotechnol. 2016;8(2):65–74.
9. Anton SD, Martin CK, Han H, et al. Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite. 2010;55(1):37–43.
10. Misra H, Soni M, Silawat N, Mehta D, Mehta BK, Jain DC. Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats. J Pharm Bioallied Sci. 2011;3:242-8.
11. Ong KW, Hsu A, Tan BK. Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by ampk activation. Biochem Pharmacol. 2013;85(9):1341-

51.
12. Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol. 2016;12(6):357-70.
13. Kim SM, Imm JY. The Effect of chrysin-loaded phytosomes on insulin resistance and blood sugar control in type 2 diabetic db/db mice. Molecules. 2020;25(23):5503.
14. Dewi NLKAA, Prameswari PND, Cahyaningsih E, Megawati F, Agustini NPD, Juliadi D. Review : Pemanfaatan Tanaman Sebagai Fitoterapi pada Diabetes Mellitus. Jurnal Integrasi Obat Tradisional. 2022;2(1):2963–2161.
15. Kumar A, Aswal S, Semwal RB, Chauhan A, Joshi SK, Semwal DK. Role of plant-derived alkaloids against diabetes and diabetes-related complications: a mechanism-based approach. Phytochemistry Reviews. 2019;18(5):1277–1298.
16. Muhammad I, Rahman N, Gul-E-nayab, Nishan U, Shah M. Antidiabetic activities of alkaloids isolated from medicinal plants. Brazilian Journal of Pharmaceutical Sciences. 2021;57.
17. Singh P, Alex JM, Bast F. Insulin receptor (IR) and insulin-like growth factor receptor 1 (IGF-1R) signaling systems: novel treatment strategies for cancer. Medical Oncology. 2014;31(1).
18. Knudsen L, Hansen BF, Jensen P, Pedersen TÅ, Vestergaard K, Schäffer L, Blagoev B, Oleksiewicz MB, Kiselyov VV, De Meyts P. Agonism and antagonism at the insulin receptor. PLoS One. 2013;7(12):e51972.
19. Castro DFP, Bernardo NJLC, Matias FBR. *Stevia rebaudiana* Leaf Extract Reduces Blood Glucose and Visceral Fat Accumulation in Alloxan-Induced Diabetic Mice. 2021;10(5):e3347.
20. Mutmainah, Martono Y, Puspitaningrum I, Kusmita L. Leaf Extract Microencapsulation of *Stevia rebaudiana* Bert Using Inulin-Chitosan as Anti-Diabetes Diet. 2020.
21. Hussain F, Hafeez J. Therapeutic Attributes of *Stevia rebaudiana* Leaves in Diabetic Animal Model. RADS J Biol Res Appl Sci. 2021;12(1):1-7.
22. El-Hadary A, Sitohy M. Safely effective hypoglycemic action of stevia and turmeric extracts on diabetic Albino rats. J Food Biochem. 2020;00:e13549.
23. Eidangbe, George O. Andrological Potential of Aqueous Leaf Extract of *Stevia rebaudiana* in Male Wistar Rats. Journal of Complementary and Alternative Medical Research. 2024;25(12):122-32.
24. Rashid AH, Mohammed MJ, Hussein FF. The Effect of Dried Stevia Leaves on Some Biochemical. Characteristics in the Blood of Diabetic Rats. IOP Conf. Series: Earth and Environmental Science. 2024;1371:062011.
25. Gupta E, Mohammed A, Mishra N, Purwar S, Rizvi SI, Sundaram S.Antioxidant and anti-diabetic potential of rebaudioside A and a mixture of steviol glycosides in alloxan-induced diabetic rats. Indian Journal of Natural Products and Resources. 2021;12(3):391-399.
26. Aissaoui O, Terki L, Ameur SA, Bitam A. In vivo evaluation of antioxydant potential and antihyperglycemic effect of *Stevia rebaudiana* Bertoni. Agronomy Research. 2024;22(1):33-51.
27. Vílchez LFV, Ascencion JH, Dooley TP. GlucoMedix®, an extract of *Stevia rebaudiana* and *Uncaria tomentosa*, reduces hyperglycemia, hyperlipidemia, and hypertension in rat models without toxicity: a treatment for metabolic syndrome. BMC Complementary Medicine and Therapies. 2022;22:62.
28. Abdillah S, Lestari AG, Monika P, Sumaryono W, Hisyam K. Preliminary Analysis of the Antidiabetic Activity of Indonesian Polyherbal Formulation. Journal of Natural Remedies. 2021;21(3):219-223.
29. Khalid M, Alkaabi J, Khan MAB, Adem A.. Insulin signal transduction perturbations in insulin resistance. Int J Mol Sci. 2021;22(16):8590–8606.
30. Li Y, Pan H, Zhang X, Wang H, Liu S, Zhang H, Qian H, Wang L, Ying H.. Geniposide improves glucose homeostasis via regulating foxo1/pdk4 in skeletal muscle. J Agric Food Chem. 2019;67(16):4483–

4492.
31. Sharma R, Kumari M, Prakash P, Gupta S, Tiwari S.. Phosphoenolpyruvate carboxykinase in urine exosomes reflect impairment in renal gluconeogenesis in early insulin resistance and diabetes. Am J Physiol Renal Physiol. 2020;318(3):F720–F731.
32. Mastrototaro L, Roden M.. Insulin resistance and insulin sensitizing agents. Metabolism. 2021;125:154892.
33. Sheikh Y, Chanu MB, Mondal G, Manna P, Chattoraj A, Deka DC, Talukdara NC, Borah JC. Procyanidin A2, an anti-diabetic condensed tannin extracted from *Wendlandia glabrata*, reduces elevated G-6-Pase and mRNA levels in diabetic mice and increases glucose uptake in CC1 hepatocytes and C1C12 myoblast cells. Royal Chem Soc. 2019;9:17211-17219.
34. Faizah S, Eriani K, Rosnizar. Effectiveness test of *Diploknema oligomera* extracts to the decrease in glucose levels in alloxan-induced BALB/c male mice. Nusantara Bioscience. 2023;15(2):143-148.
35. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced Diabetes. Diabetologia. 2008;51(2):216-26.
36. Yohanna FT, Haruna WC, Yerima TS, Sodipo OA, Aliyu F. 2018. Phytochemical screening and Antidiabetic effect of ethanol extracts of *Leptadenia hastata* plant (Pers.) Decne) in alloxan-induced diabetic rats. Nigerian Journal of Pharmaceutical and Biomedical Research. 2018;3(1):22-27.
37. Jain DK, Arya RK. Anomalies in alloxan-induced diabetic model: It is better to standardize it first. Indian Journal of Pharmacology. 2011;43(1):91.
38. Chougale AD, Panaskar SN, Gurao PM, Arvindekar AU. Optimization of Alloxan Dose is Essential to Induce Stable Diabetes for Prolonged Period. Asian Journal of Biochemistry. 2007;2(6):402-408.
39. Aybar MJ, Sanchez Riera AN, Grau A, Sanchez SS. Hypoglycemic effect of the water extract of *Smallanthus sonchifolius* (yacon) leaves in normal and diabetic rats. J. Ethnopharmacol. 2001;74:125–132.
40. Petchi RR, Parasuraman S, Vijaya C. Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Linn.) in streptozotocin-induced diabetic rats. J. Basic Clin. Pharm. 2013;4:88–92.
41. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. Avicenna J Med. 2020;10(4):174–188.