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Unveiling *Stevia rebaudiana*: origins, composition, and health implications

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Abstract: *Stevia rebaudiana* is an important plant known for its high-quality, non-caloric sugar substitute properties, making it a beneficial option for metabolic disorders. Diabetes and obesity are interlinked and are a surging health issue around the globe. This review article aims to synthesize existing literature to offer a comprehensive understanding of stevia, its origin, nutritional composition, and health implications contributing to the body of knowledge in this area. Research methods employed for this review included a systematic literature search in reputable databases such as PubMed and Web of Science to gather and synthesize relevant findings. This review focuses on the composition of stevia including nutritional profiling) and phytochemical profiling, its metabolic absorption and health implications, emphasizing its role in weight management and glucose regulation. Stevia leaf drying technique for extending the shelf life of the leaves and extraction of steviol glycosides has been thoroughly discussed. This review primarily envisages the mechanisms involved in the therapeutic effects of *Stevia rebaudiana* components and their contribution toward reducing metabolic diseases.

Keywords: diabetes, health benefits, phytochemicals, stevia

1. Introduction

Stevia rebaudiana (Bertoni) is a member of the Asteraceae family, which encompasses around 950 genera. Centuries ago, the indigenous people of Paraguay used the leaves of this small, herbaceous, semi-bushy, perennial shrub to sweeten their bitter beverages (Rashid et al., 2021). The Guarani Indians utilized this plant for over 1500 years. Dr. Moises Santiago Bertoni first identified the plant in Paraguay in 1888. In 1905, the scientific name Stevia rebaudiana was given in honor of Dr. Rebaudi, a Paraguayan chemist. It is noted that there are approximately 150 species within the Stevia genus, including *Stevia dianthoidea*, *Stevia phlebophylla*, *Stevia anisostemma, Stevia bertholdii, Stevia crenata, Stevia enigmatica, Stevia eupatoria, Stevia lemmonii, Stevia micrantha, Stevia ovata, Stevia plummerae, Stevia rebaudiana, Stevia salicifolia, Stevia serrata,* and *Stevia viscida*. *Stevia rebaudiana* is known for being the best sweetener (Gupta et al., 2013).

Stevia rebaudiana has been utilized globally for various applications. The Guarani tribes traditionally used *Stevia rebaudiana* as a sweetener in their herbal infusions. Japan pioneered in Asia to introduce steviol glycosides into the food and pharmaceutical industries. This led to the spread of its cultivation to countries such as China, Malaysia, Singapore, South Korea, Taiwan, and Thailand (Lemus-Mondaca et al., 2012). Steviol glycosides have been employed as a substitute for sucrose and in the treatment of conditions like diabetes mellitus, obesity, hypertension, and dental caries (Pól, Hohnová and Hyötyläinen, 2007). Research indicates that steviol glycosides offer therapeutic benefits, including antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, diuretic, and immunomodulatory effects (Chatsudthipong and Muanprasat, 2009). Additionally, stevia leaves possess functional and sensory attributes that surpass those of many other sweeteners (Pól, Hohnová and Hyötyläinen, 2007).

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Sweetness is one of the basic taste sensations that significantly influences human food preferences and dietary habits (Li et al., 2020). Sugars, straightforward carbohydrates such as sucrose (table sugar), supply immediate energy and enhance the taste of food. However, high consumption of sugar is associated with numerous health issues, including obesity, type 2 diabetes, and cardiovascular diseases (Nguyen et al., 2023). This concern has driven the quest for alternative sweeteners that can provide sweetness without the adverse health effects linked to sugar. The search for sugar substitutes has driven the investigation into various natural sources of sweetness. Over the past few decades, artificial sweeteners like aspartame and sucralose have been commonly used. However, safety concerns and potential long-term health effects have reignited interest in plant-derived natural sweeteners (Soares et al., 2021).

Diabetes and obesity are both major concerns for global health, and they are closely related. Obesity increases the risk of having diabetes, and it can also contribute to the progression of the disease as well as cardiovascular issues (Verma and Hussain, 2017). While weight loss is widely recognized as a beneficial means of preventing and managing diabetes, it is challenging for those with type 2 diabetes due to various metabolic and psychological factors (Ibrahim et al., 2018). For some individuals, lifestyle changes alone may not be sufficient for weight loss, and other options like pharmacotherapy must be considered. Nonetheless, many conventional glucose-lowering medications can cause weight gain (Van Gaal and Scheen, 2015). Diabetes is a non-communicable and metabolic disorder characterized by hyperglycemia, a condition in which either the pancreas is unable to produce insulin, or the body is unable to utilize the produced insulin which as a result increases blood glucose level (Shahid et al., 2021). Insulin, a hormone produced by β-cells of the pancreas plays a key role in the metabolism of carbohydrates and fats. It allows glucose to enter the cells from the bloodstream (Qaid and Abdelrahman, 2016). The insulin resistance or inability of the cells to respond to insulin can lead to a rise in blood glucose levels. This condition is known as hyperglycemia which indicates diabetes. The signs and symptoms of diabetes occur gradually, including polyuria (excess urination), polydipsia (excess thirst), polyphagia (excess hunger) and lethargy, blurry vision, irritability, mood swings, and unexpected weight loss (Roglic, 2016).

Type 2 Diabetes or Non-Insulin Diabetes Mellitus is the most prevalent type of diabetes accounts for more than 90.0% of the people suffering and is also ranked as the sixth leading cause of disability (Chatterjee, Khunti and Davies, 2017). It is a metabolic condition characterized by increased levels of blood glucose resulting from defective secretion of insulin or ineffective insulin action. It is considered adult-onset diabetes or insulin-independent diabetes mellitus. In type 2 diabetes, insulin resistance occurs, and cells of the body do not respond properly to the insulin produced (Shawahna, Samaro and Ahmad, 2020). The primary causes of Type 2 diabetes include genetics and overweight or obesity. In Type 2 diabetes insulin is present inside the blood but insulin receptors lose their sensitivity and cannot sense available insulin due to which glucose channels remain closed and glucose cannot penetrate the cells (Davies et al., 2018).

Globally 422 million people are diabetic. More than 95.0% of diabetic individuals are type 2 diabetic and Type 1 diabetes in children and adolescents is above 1.1 million (Manjunatha and Jayashree, 2019). Diabetes is considered the ninth leading cause of mortality with a death count of 1.5 million deaths per year. The total number of people with diabetes will increase to 578 million by the end of 2030 and may increase to 700 million by the end of 2045 (Lemieux, 2020). The International Diabetes Federation (IDF) in its ninth report stated that the prevalence of diabetes in Pakistan has reached 17.1% in the adult population which is 148.0% higher than previous reports and it is estimated that by the end of 2019, the diabetic adult population will increase over 19 million (Saeedi et al., 2019).

The diagnostic criteria for pre-diabetes and diabetes are recommended by the American Diabetes Association (Schwartz et al., 2017). It includes a non-fasting blood glucose test (HbA1c), Random blood glucose test, Fasting blood glucose test, and Glucose tolerance test (GTT) (Olson et al., 2010). HbA1c (glycated hemoglobin) is the test that measures average blood glucose levels over the past 3 months. The normal results for the HbA1c test are between 4.6 and 5.6%. 5.7-6.4%, in terms of millimole/mole (mmol/mol) (between 39 and 47 mmol/mol) indicates pre-diabetes, and ⩾6.5% (⩾47mmol/mol) indicates diabetes (Li et al., 2018).

Numerous pharmaceutical drugs have been developed for managing and treating type 2 diabetes mellitus, with metformin being the primary choice. Metformin functions by enhancing insulin sensitivity, thereby facilitating increased glucose uptake in the liver (Overbeek et al., 2017). Glitazones were utilized in the past to improve insulin sensitivity in muscles but their withdrawal from the market was necessitated due to severe side effects (Rubenstrunk et al., 2007). Antidiabetic agents such as Sodium-glucose cotransporters (SGLT-2) inhibitors and Dipeptidyl Peptidase IV (DPP-4) inhibitors have been introduced to manage hyperglycemia. SGLT-2 inhibitors

exert their antidiabetic effects by blocking SGLT-2, which enhances glucose excretion in urine, consequently reducing blood glucose levels. On the other hand, DPP-4 inhibitors promote insulin secretion and inhibit the breakdown of Glucagon-like peptide 1 (GLP-1), an insulinotropic hormone, resulting in decreased blood glucose levels (Scheen, 2015). Certain medications demonstrate insulin-resistance-reducing properties and may decrease the reliance on insulin secretagogues like meglitinides and sulfonylureas. However, it is important to note that none of the current drugs offer a complete cure for diabetes, and most of them are associated with potential side effects and high costs (Anker, Rafiq and Jeppesen, 2019).

Obesity is a metabolic disturbance where there is an excess accumulation of body fat, which is affected by both genetic and environmental factors that are challenging to manage with dietary interventions alone (Schwartz et al., 2017). Adipose tissue is an endocrine organ where fat cells within it are endocrine cells. They secrete various products, including cytokines, lipids, metabolites, and coagulation factors (McGown, Birerdinc and Younossi, 2014). Excessive adiposity or obesity leads to increased levels of circulating fatty acids and inflammation, which results in insulin resistance and, ultimately, type 2 diabetes (Siddiqui et al., 2018). The 2nd National Diabetes Survey conducted in Pakistan during 2016-2017 concluded the overall prevalence of generalized obesity in Pakistan was 57.9%. The occurrence of generalized obesity was recorded at 42.0% among males and 58% among females, using the cutoffs established by the World Health Organization (WHO) (Qazi et al., 2023). Meanwhile, the rate of central obesity was 73.1%, with 37.3% among males and 62.7% among females respectively. The province of Punjab had the highest prevalence of generalized obesity at 60%, followed by Khyber Pakhtunkhwa at 59.2%. In terms of abdominal obesity, Baluchistan had the highest prevalence at 82.1%, followed by Punjab at 73.3%. It was noted that there was a high association between obesity (generalized and abdominal), diabetes, hypertension, and dyslipidemia (Basit et al., 2021).

Being overweight is a major risk factor for type 2 diabetes, but not all obese populations develop this condition (Wilding, 2014). Latest research has revealed the existence of a connection between obesity and type 2 diabetes, which involves various factors including pro-inflammatory cytokines like tumor necrosis factor, interleukin-6, insulin resistance, disordered fatty acid metabolism, and cellular biochemical reactions such as dysfunction of mitochondria, and stress in the endoplasmic reticulum (McCracken, Monaghan and Sreenivasan, 2018). The interactions between these factors are complex, and the significance of each is not entirely clear. Further genetic studies could help identify more ways for obesity and diabetes and identify new treatments. Doctors commonly prescribe glucose-reducing medications that can lead to weight gain, so it is essential to consider the compromise between glycemic control and body weight (Lipska et al., 2016). This is particularly important because there is growing evidence that even modest weight loss can improve glycemic control and lower the chances of diabetes through behavioral interventions, obesity medications, and bariatric surgery (Eckel et al., 2011).

Diet is considered the key variable factor for numerous chronic diseases. A poor-quality diet and food choices increase the risk of type 2 diabetes (WHO, 2003). Abnormal dietary patterns are associated with the onset and progression of cardiovascular diseases, hypertension, cancers, and type 2 diabetes. Once established, food selection and diet play an important role in the management of Type 2 diabetes (Conklin et al., 2016). Dietary management and physical activity are key elements in preventing and managing Type 2 diabetes and obesity. Emphasizing the acceptance and maintenance of physical activity is required for managing blood glucose levels and health in individuals with type 2 diabetes (Sami et al., 2017).

Recommendations and precautions should be tailored/ to individual characteristics and good health status. Physical activity has been shown to improve blood glucose control in Type 2 diabetes (Lambert and Bull, 2014). A study has found that structured lifestyle interventions, consisting of at least 150 min per week of physical activity in addition to dietary changes and modest weight loss, are effective in managing Type 2 diabetes. This approach was found to overcome the risk of type 2 diabetes up to 58% in highly high-risk populations (Colberg et al., 2016).

Stevia leaves possess superior sensory and functional properties compared to many other high-potency sweeteners, making them a promising natural sweetener for the expanding food market (Goyal et al., 2010). Known for their beneficial effects on human health, Stevia leaves have garnered significant research interest. The leaves of *S. rebaudiana* offer various medicinal applications, including antimicrobial (Satishkumar et al., 2008), antiviral (Kedik et al., 2009), antifungal (Silva et al., 2008), antihypertensive (Chan et al., 1998; Lee et al., 2001; Hsieh et al., 2003), antihyperglycemic (Jeppesen et al., 2002; Benford et al., 2009), antitumor (Satishkumar et al., 2008), anti-inflammatory, antidiarrheal, diuretic, anti-human rotavirus (Das et al., 1992; Takahashi et al.,

2001), anti-HIV (Takahashi et al., 1998), hepatoprotective (Mohan and Robert, 2009), and immunomodulatory effects (Pól, Hohnová and Hyötyläinen, 2007; Chatsudthipong and Muanprasat, 2009).
Excessive sugar consumption has raised significant health concerns, increasing the risk of chronic diseases for future generations. A large portion of an individual's caloric intake often comes from sugar, with many unaware of its detrimental effects. Recent studies have introduced better alternatives, and Stevia has emerged as a highly researched substitute worldwide. Although some benefits of Stevia have been identified, many more remain to be discovered. As people learn about its functionality, they gradually incorporate Stevia into their diets (Khan et al., 2019). Many countries now recognize the harmful effects of sugar on health and are increasingly turning to Stevia as a natural, safe alternative. Stevia serves as a versatile sweetener, used in a wide range of consumer products, including soft drinks, toothpaste, ice creams, confectionery, and ointments. Its numerous benefits extend beyond sweetening foods, as it can also be used to manufacture medicines (Yadav and Guleria, 2012). Stevia outperforms sugar in various aspects: it is cheaper, more efficient, and offers numerous health benefits. It tastes sweeter than sugar and can be easily integrated into daily routines. Stevia finds applications in skin care products, dental products like toothpaste and mouthwashes, edible products, sanitary products, flavoring products, and sweetening products. Although current use is limited, ongoing and future research will likely encourage a significant shift from sugar to Stevia. Known as herbal sugar and a magical sweetener, *Stevia rebaudiana* promises a valuable future with countless health benefits. According to the WHO, Stevia is expected to gain a larger share of the sweetener market, positioning it as the future of sugar (Wang et al., 2014).
The existing research on Stevia and diabetes or obesity is limited and inconclusive, highlighting the need for more rigorous investigation. Therefore, this review article aims to investigate the potential health benefits of Stevia in type 2 diabetic patients and other metabolic disorders by exploring Stevia's nutritional profile, potential benefits, and limitations. This study literature can provide insights into its role as an alternative therapy for individuals with diabetes who face challenges in achieving weight loss.

2. Materials And Methods

The methodology used in this study included a comprehensive literature review. The search was conducted by searching articles from Google Scholar (http://www.Googlescholar.com), ScienceDirect (https://www.sciencedirector.com/), Scopus (http://www.scopus.com), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed) by searching keywords such as "*Stevia rebaudiana*", "Effect of stevia", "Mechanism of stevia". The search was conducted in English. Findings from relevant contexts and research journals are presented in an instructive manner. Articles published between 2003 and 2023 were included in the analysis.

3. *Stevia rebaudiana* Bertoni

Stevia rebaudiana Bertoni is an Asteraceae family plant with branches and a bushy appearance. The tree or shrub is indigenous to northeastern Paraguay (Lemus-Mondaca et al., 2012). China and Southeast Asia have also raised the plant. For several decades, Japan, Korea, and Brazil have utilized stevia sweeteners, which are unprocessed extracts from their leaves, to sweeten beverages and dishes (Madan et al., 2010).
Products made from *Stevia rebaudiana* are utilized as dietary supplements in the United States of America. Stevioside (triglucosylated steviol) and rebaudioside A (tetraglucosylated steviol) along with the additional ingredients rebaudioside C and glucoside A, are the two primary components of stevia the sweetener, which is diterpene derivative steviol (ent-13-hydroxykaur- 16-en-19-oic acid) glycosides (De, Mondal and Banerjee, 2013). Both stevioside and rebaudioside A are glycosides found in stevia that provide no calories (Mathur et al., 2017). Along with glycosides, stevia also includes a variety of volatile oils, tannins, stigmasterol, labdane diterpenes, and triterpenes. Together, these compounds make up around 5.0–10.0% of the dry leaf weight. Dulcoside A as well as rebaudioside C lack 200–300 times the sweetening capacity of table sugar, sucrose that only stevioside and rebaudioside A possess (Chaturvedula and Prakash, 2011).

4. Composition

4.1 Nutritional profiling of stevia

Nutritional profiling of stevia has been estimated in several researches. The proximate analysis of stevia was estimated per 100 grams (g) of dried sample including the moisture content 5.5 g, proteins 16.2 g, fats 3.8 g, ash 8.2 g, fiber 7.9 g and carbohydrates 58.8 g (Befa, Gebre and Bekele, 2020).
The mineral analysis of dried stevia leaves was estimated through an Atomic Absorption Spectrophotometer. The results expressed in milligrams (mg) per 100 grams showed that stevia contains calcium 470.3 mg,

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phosphorus 27.0 mg/100 g (Gupta and Purwar 2015), sodium 102.9 mg, magnesium 347.4 mg, iron 297.9 mg, zinc 3.7 mg, copper 1.1 mg, and manganese 9.4 mg/100 g (Befa, Gebre and Bekele, 2020).

4.2 Phytochemical Profiling of Stevia

The estimation of phytochemical profiling including Total Phenolic content (TPC), Total Flavonoids content (TFC), and Ferric reducing Antioxidant Power (FRAP) assay of stevia involved the preliminary steps including preparation of the sample extract through maceration of 1 gram of dried stevia leaves in 25 ml methanol for 24 hours (Mandal and Madan, 2013).

The prepared dried stevia extract was then used for the estimation of phytochemical profiling. Stevia contains the TPC 18.6 mg of gallic acid equivalent per gram, TFC 3.91 mg quercetin equivalent/gram of dry weight, and FRAP 56.6 mmol of Fe2+ per gram of dry weight (Shahu et al., 2022). The procedure of phytochemical profiling is shown in (Figure 1).

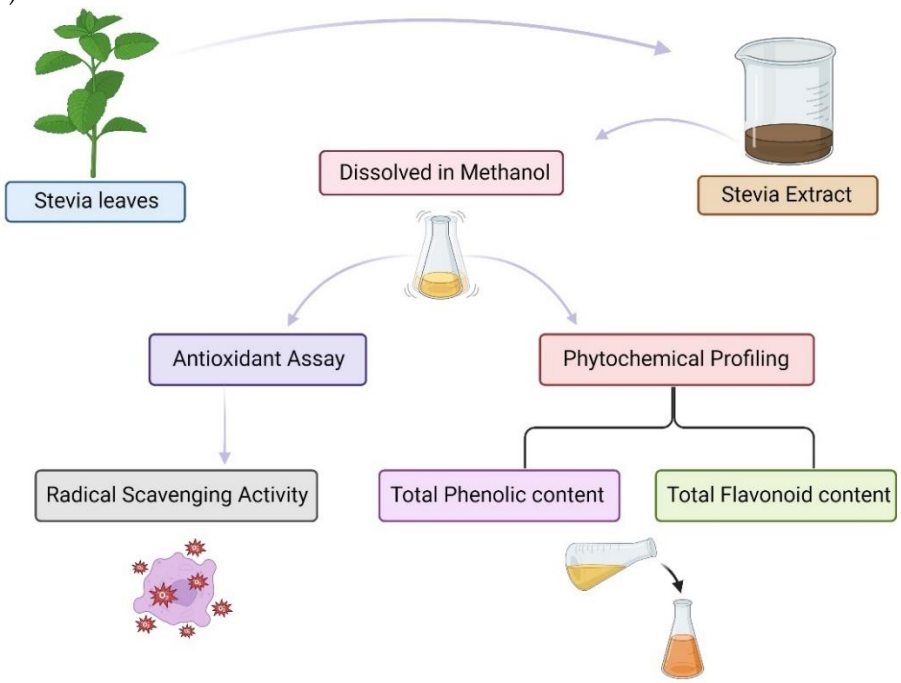

Figure 1. Phytochemical profiling of *Stevia Rebaudiana*

4.3 Extraction of Stevia glycosides

Stevia leaf drying is a useful technique for extending the shelf life of the leaves. But drying alters the product, mostly in ways that affect the fragrance (Babu et al., 2018). It is unclear what chemicals exactly cause these alterations. Depending on the volatile molecule and the product being dried, a certain drying method's impact on the release or retention of volatile compounds is unpredictable. The most common treatment, by far, is drying, but it must be done properly to retain as much of the raw materials' flavor and color as possible (Ibrahim et al., 2007).

Ashwell mentioned in his study about the extraction of steviol glycosides from stevia. Stevia is purified into stevia leaf extract through a series of stages that start with the harvested, raw plant material and conclude with the finished product (Ashwell, 2015). The leaves must first be dried before being steeped in boiling water. The liquid extract is filtered and purified using either water or food-grade alcohol, depending on the situation (Samsudin and Aziz, 2013). In cases where alcohol is used, it is later evaporated leaving no detectable levels of it in the finished material. In some situations, several techniques could be applied (Wang et al., 2020). The procedure of extraction of steviol glycosides is explained in (Figure 2).

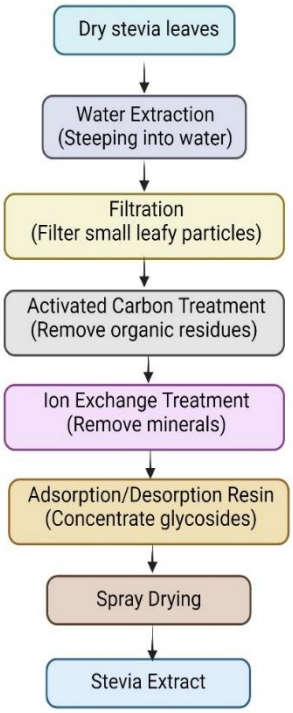

Figure 2. Processing steps to obtain stevia glycosides leaf extract from the stevia plant

Carakostas and colleagues reported the early formulations of stevia extract which is known as a licorice flavor with a sweet and bitter aftertaste, which hindered its extensive growth in the United States in the 1990s (Carakostas et al., 2008; Prakash et al., 2008). Some off tastes were caused by essential oils, tannins, and flavonoids present in the crude extracts, efforts were made to purify extracts and characterize steviol glycosides chemically (Samuel et al., 2018).

Sweetness in stevia is derived from steviol glycosides, which are composed of steviol as its central component. These glycosides contain various groups of glycosides attached to the steviol core to create a range of sweet compounds (Gerwig et al., 2016). Steviol glycosides do not degrade in the upper gastrointestinal tract. However, when they reach the colon, gut bacteria remove the glucose units from the glycosides, converting them into steviol (Prakash and Chaturvedula, 2018). The liver is primarily responsible for metabolizing steviol, which results in the formation of steviol glucuronide, which is eliminated in the urine, and steviol is subsequently absorbed by the body through the portal vein (Meesschaert et al., 2021). Research suggests that the body does not accumulate stevia; instead, it is metabolized as it moves through the body. Since fermentation in the glucose unit produces 2 calories per gram of energy, stevia is considered to have no caloric value (Gardena et al., 2003).

4.4 Safety of steviol glycosides

The safety of steviol glycosides has been assessed by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) in 2000, 2004, 2005, 2007, and 2009 (EFSA, 2020). Stevia has also been Generally Recognized as Safe (GRAS) by regulatory agencies, including the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). During these reviews, an Acceptable Daily Intake for steviol glycosides was established, with a maximum limit of 4 mg per kilogram (kg) of body weight per day, expressed as steviol equivalents (Magnuson et al., 2016).

Research conducted on human subjects has revealed that the consumption of steviol glycosides up to 1000 mg per day for a person is safe and does not cause any adverse health effects (EFSA, 2020). The amount mentioned is equivalent to 16.6 mg per kg body weight per day for a person weighing 60 kg. Therefore, it is equivalent to roughly 330 mg or 5.5 mg steviol equivalent/kg body weight/day (Blaauboera et al., 2016). These findings are consistent for individuals with normal glucose metabolism as well as those with type 2 diabetes (Samuel et al., 2018). Many South American and Asian countries already use stevioside as a food sweetener. Stevioside and Stevia extracts have been shown in several trials to have hypoglycemic and hypotensive effects, especially in those with type 2 diabetes and hypertension (Chen et al., 2005; Jeppesen et al., 2003).

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4.5 Metabolic absorption of steviol glycosides

Absorption, metabolism, and excretion of steviol glycosides have undergone extensive examination by numerous scientific authorities and experts, such as EFSA (EFSA, 2020). Steviol glycosides are resistant to digestion in the upper gastrointestinal tract but can be broken down by colonic microbiota through the enzymatic cleavage of glycosidic linkages (Pang, Goossens and Blaak, 2021). This enzymatic process removes the sugar components, resulting in the absorption of the steviol backbone into the systemic circulation. Subsequently, the steviol undergoes glucuronidation in the liver and is excreted through urine by humans and through feces by rats (Magnuson et al., 2016).

An earliest In-vitro study has demonstrated human saliva, salivary amylase, pepsin, pancreatin, pancreatic amylase and jejunal brush border enzymes of mice, rats, and hamsters cannot break the glycosidic bonds within stevioside (Samuel et al., 2018). The gut microbiota of humans, hamsters, and rodents is capable of degrading stevioside into steviol. Emerging of stevioside and Rebaudioside A (Reb. A) along human fecal microbiota has revealed complete hydrolysis to steviol within 10 and 24 hours, respectively (Purkayastha et al., 2015). The released sugar components are not absorbed and are likely utilized as an energy source by the gut microbes, ultimately contributing to the zero-calorie nature of steviol as a sweetener (Wang et al., 2023). The mechanism of absorption of steviol glycosides is explained in (Figure 3).

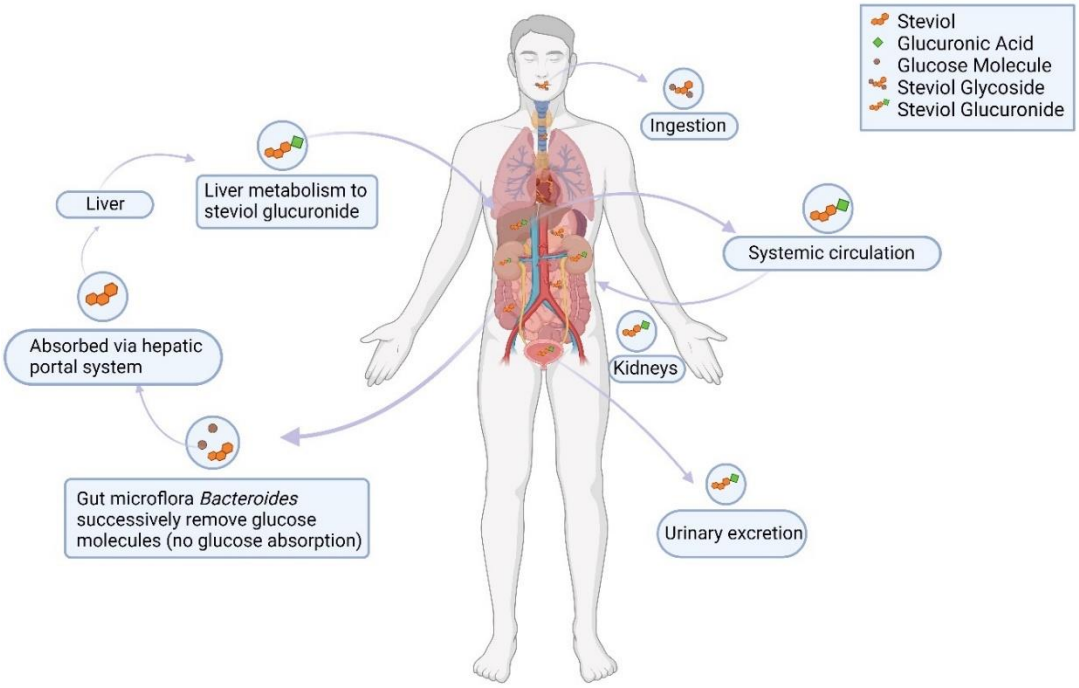


Figure 3. Mechanism of absorption of steviol glycosides in the human body

5. Health Implications

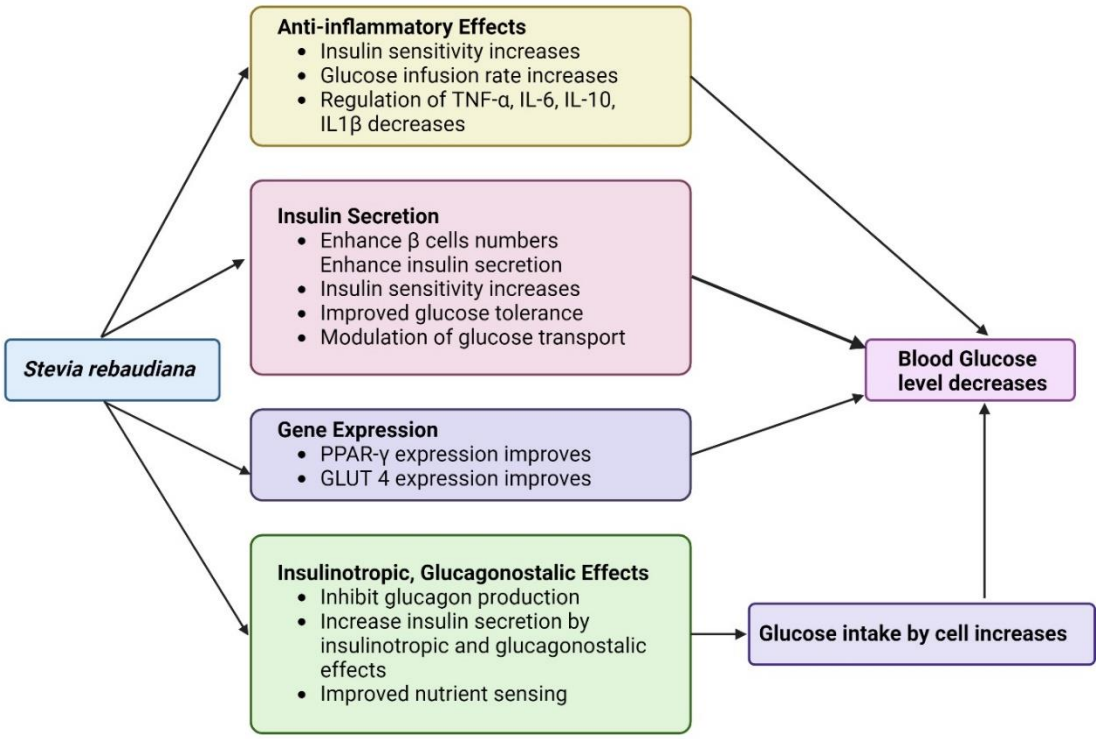
5.1 Antihyperglycemic effects of stevia extract

The active ingredients in stevia have been shown to lower blood glucose levels. Stevioside, the main compound in stevia, lowers blood glucose levels through several mechanisms, including increased insulin secretion and sensitivity, as well as decreased glucagon metabolism (Carrera-Lanestosa et al., 2017).

Ritu and Nandini proved in their study that stevia has several positive benefits, including the ability to reduce blood sugar levels, manage diabetes, prevent dental cavities, control weight, heal wounds and blemishes, lower blood pressure, and modulate the immune system (Ritu and Nandini, 2016). It also has anti-fungal and antibacterial characteristics. It is non-toxic and harmless for diabetics, and long-term usage of it may help individuals with long-term diabetes prevent cardiovascular illnesses (Singh and Rao, 2005).

The direct effect of stevioside on pancreatic beta cells to increase insulin secretion and enhance their function in glucotoxicity may be the cause of the potential glucose-lowering effect (Jehan and Ashoush, 2008). As it

improves the initial insulin response, and concurrently suppresses the levels of glucagon, it can also have a hypoglycemic effect. It is highly advised for usage by diabetics (Talevi, 2022). It causes hypoglycemia in diabetics by decreasing glycogenolysis and gluconeogenesis processes by absorbing the glucose in the duodenum portion. It may also be used effectively and safely to replace sweeteners (Assaei et al., 2016). The actions of *Stevia rebaudiana* on the blood glucose levels are shown in (Figure ٤).



In a study with 16 participants, the impact of *Stevia rebaudiana* leaf extracts on glucose tolerance was investigated over 3 days. Aqueous extracts were administered at 6-hour intervals. Glucose tolerance tests were conducted at the initial and final administration. A second group ingested an aqueous arabinose solution. Results showed a positive influence on glucose tolerance, with a significant reduction in plasma glucose levels during the test and after overnight fasting in all participants, suggesting a potential benefit for glucose metabolism (Curi et al., 1986).

An insignificant decline was observed in both HbA1c and fasting glucose levels among the individuals with type 2 diabetes following a 16-week treatment with Reb. A about 1000 mg/day. A marginal increase in HbA1c levels was observed in both the intervention and group with placebo, with no discernible change in HbA1c noted between the Reb. A and placebo groups. Additionally, there was a significant increase in fasting insulin within the Reb. A group when compared to the placebo group. Alterations from the baseline to the treatment did not exhibit a significant distinction between the two groups (Maki et al., 2009). The research concluded that a water extract derived from stevia leaves at doses of 3.125 mg/kg BW, 6.25 mg/kg BW, and 12.5 mg/kg BW exhibited significant reductions in blood glucose levels (Lestari et al., 2019). Refer to (Table ١) for the studies references performed to evaluate the beneficial effects of stevioside.

Table 1: Health Benefits of Stevioside in Humans

Subjects	Trials duration	Treatment/Dosage	Results	References
Diabetic and obese males and females	4 hours	1000 mg/day stevioside	Decline in random blood glucose levels	(Gregersen et al., 2004)
Type 2 diabetic males and females	3 months	1500 mg/day stevioside	No effects on blood glucose, HbA1c, and triglycerides levels	(Jeppesen et al., 2003)
Males and females with or without type 1 or type 2 diabetes	3 months	750 mg/ day stevioside	No effects on blood glucose, body mass index, HbA1c, and cholesterol levels	(Barriocanal et al., 2008)
Males and females with hypertension	1 year	750 mg stevioside	Lowered blood pressure but shown no effects on blood glucose and lipid profile	(Chan et al., 2000)
Type 2 diabetic males and females	16 weeks	1000 mg rebaudioside	No changes in blood pressure and blood glucose levels	(Maki et al., 2009)
Obese and healthy males and females	3 days	400 g stevia incorporated in tea	Improved appetite, normal blood	(Anton et al., 2010)

		and crackered with cream cheese	glucose levels, and insulin levels	
University students	1 week	Stevia incorporated as a sweetener in beverages and foods	Improved normal weight and nutritional status	(Agüero et al., 2015)
Males and females with hyperlipidemia	90 days	200 mg stevioside	No improvement in blood lipid levels	(Silva et al., 2006)
Males and females with or without type 1 or type 2 diabetes	3 months	750 mg/ day stevioside	No beneficial effects on blood cholesterol levels	(Barriocanal et al., 2008)
Type 2 diabetic males and females	3 days	1 gram stevia	Decrease in blood lipid profile	(Ritu and Nandini, 2016)

(mg= milligrams, HbA1c= glycated hemoglobin)

5.2 Weight managing effects of stevia

Anton and colleagues conducted a randomized clinical study to evaluate the impact of two preloads on food consumption and levels of satiety in both lean and obese individuals over three days. The preloads contained Stevia 580 kcal, aspartame 580 kcal, or sucrose 986 kcal and were taken for 20 minutes before the ad libitum meals twice daily (Anton et al., 2010). The study found that the stevia group showed a significant decrease in overall food intake as contrasted to sucrose sucrose-taking group, including the preloads (Farhat, Berset and Moore, 2019). Preload calories were excluded from analysis, there was no difference in food consumed between the groups. The researchers could not observe any changes in satiety levels between the groups at the time, suggesting participants have not compensated for consuming more during lunch or dinner (Anker, Rafiq and Jeppesen, 2019).

In cohort trial encompassing 1454 participants who reported regular intake of artificial sweeteners over a long time, revealed a noteworthy increase in BMI among users compared to nonusers. The study showed an independent relation between low-calorie sweetener use and a heightened incidence of obesity in the abdominal area, broad waist, and increased body weight. Baseline characteristics indicated that artificial sweetener consumers exhibited larger BMI values and larger waist circumference than non-users. This observation may reflect weight management efforts in this group through the utilization of artificial sweeteners. Conversely, several studies exploring the connections between artificial sweeteners and obesity, diabetes, and cardiovascular disease couldn't be solely related to elevated baseline waist circumference or BMI (Rojas et al., 2018).

The study on the impact of stevia extracts on diet-induced obesity and associated lipid abnormalities while contrasting with the adverse effects of a sucrose diet concluded that stevia extract supplementation contributes to reducing weight gain, as well as decreased levels of serum and liver triglycerides. The study hinted at a potential upregulation of genes responsible for encoding enzymes used for the oxidation of fatty acids in the

liver. effectiveness of stevia extract in preventing obesity and related symptoms, such as hyperlipidemia and cardiovascular diseases (Khiraoui and Guedira, 2018).
The randomized control trial among 20 healthy subjects who consumed the cookies enriched with stevia leaf powder proved a significant decline in hunger in contrast to the control group. Importantly, all cookie variants were palatable, and no adverse effects on gastrointestinal well-being were reported, affirming their acceptability for consumption (Ahmad et al., 2018).

5.3 Effect of stevia on blood pressure

Maki and colleagues conducted a trial to investigate the effects of a steviol glycoside preparation on glucose levels and blood pressure responses during a meal tolerance test. The study involved 93 participants with normal glucose tolerance or type 2 diabetes, who consumed a steviol glycoside preparation at three different dose levels (500 mg, 750 mg, and 1000 mg) (Maki et al., 2009). The results revealed no positive differences in the Incremental Areas Under the concentration Curves (IAUC) for glucose levels, insulin, C-peptide, and glucagon levels between the steviol glycoside preparation and placebo groups, or between any of the dose levels (EFSA, 2010). Additionally, there were no significant differences in postprandial systolic or diastolic blood pressure between the steviol glycoside preparation and placebo groups (Ray et al., 2020). The authors concluded that a dose of up to 1000 mg of the prepared steviol glycoside (equivalent to 319 mg steviol) did not affect glucose homeostasis or blood pressure in people with normal glucose tolerance or type 2 diabetes mellitus (Benford et al., 2009).
Barriocanal and colleagues conducted a study to investigate the outcome of steviol glycosides on blood glucose and blood pressure among three groups of individuals through a study (Barriocanal et al., 2008). Participants in the groups were randomly assigned to either treatment (250 mg steviol glycoside) or placebo and were followed up for three months (Ulbricht et al., 2010). Results showed no significant differences in after-treatment systolic Blood Pressure (BP), diastolic BP, glucose, and HbA1c from baseline measurements, except for the placebo type 1 diabetic group where a significant difference was observed for systolic BP and glucose. It was concluded oral steviol glycosides taken as sweeteners are highly tolerated and do not show any pharmacological effect (Carakostas et al., 2008).
Wang and colleagues conducted a study to compare the Angiotensin-Converting Enzyme inhibitory activity of ethanol extracts, steviol glycosides, and protein hydrolysates with captopril. The order of ACE inhibitory activity from highest to lowest was captopril > protein hydrolysate > steviol glycosides > ethanol extract from stevia. Despite lower ACE 2 inhibitory protein hydrolysate and steviol glycoside compared with captopril, their toxicity results were good (Wang and Wu, 2019).

5.4 Effects of stevia on cancer

Khare and colleagues investigated the potential anti-cancer attributes of purified stevioside isolated from *Stevia rebaudiana* leaves in human breast cancer cells. The findings indicated that *Stevia rebaudiana* exhibits the ability to impede the growth of cancerous cell lines, specifically MDA-MB-231 and SKBR3, by inducing apoptosis and inhibiting cell proliferation. Moreover, the study revealed that purified stevioside plays a significant role by enhancing chemosensitivity in breast cancer cells to Fluorouracil (5-FU) treatment in vitro (Khare and Chandra, 2019).
In HT29 colon cancer cells, stevioside was shown to be active in promoting apoptosis by increasing the expression of MAPK (mitogen-activated protein kinase) as well as ERK and p38, which play a role in ROS-mediated apoptosis (Ren et al., 2017).
The anticancer mechanism of stevia involves the regulation of cyclin-dependent kinases (CDK), which are proteins important in cell cycle regulation and proliferation Ethanol extract of stevia exhibited anticancer activity in HeLa, HCT116, and MiaPaCa-2 cells by inhibiting CDK4 (López et al., 2016). Previous studies showed that steviol exerted an inhibitory effect on six types of human esophageal cancer cells Yati, comparable to 5-FU. Steviol pathway inhibition includes upregulation of Bax/Bcl-2 levels, activation of p21 and p53, and caspase 3-independent activation (Chen et al., 2018).

5.5 Effects of stevia on lipid profile

The research was conducted on 20 hypercholesterolemic women to investigate the impact of stevia extract consumption administered as 20 ml in 200 ml water. The findings demonstrated a reduction in blood

cholesterol, triglyceride, and LDL levels, accompanied by a desirable increase in HDL. The observed hypolipidemic effect suggests that stevia extract could play a role in lowering the risk of cardiovascular disease (Sharma, Mogra and Upadhyay, 2009).

In the diabetic cohort, the administration of stevioside over 24 weeks demonstrated improvement in both lipid profile and glycemic control as evident across various parameters including plasma glucose in fasting state, 2 hours plasma glucose, fasting serum insulin, and hemoglobin A1c (HbA1c). Additionally, stevioside exerted positive effects on serum total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein, across all intervention groups studied (Rashad et al., 2019).

The decrease in LDL levels caused by stevioside is due to its mechanism of upregulating LDL receptors and modulating cholesterol transport This upregulation of LDL receptors increases the absorption of LDL cholesterol from the blood. Furthermore, studies have shown that stevioside, as well as methanol and water extracts from stevia leaves, lower VLDL levels (Sunanda and Veena, 2014).

The daily consumption of 1 gram of stevia over 60 days among diabetic patients demonstrated a significant reduction in blood cholesterol, triglyceride, and LDL-cholesterol levels (Ritu and Nandini, 2016).

6. Conclusion

Stevia a natural sweetener obtained from the plant *Stevia rebaudiana* has gained considerable attention as a potential alternative to sugar for people with diabetes. It contains several significant phytochemicals such as Steviol, Stevioside, and rebaudioside. These compounds have beneficial effects on pancreatic tissue by increasing insulin levels and enhancing its anti-diabetic properties. It also possesses hypolipidemic, cancer-protective, and blood pressure lowering effects. Extensive research has been conducted on the plant, but further investigation is required on the pharmacological properties of rebaudioside, as well as the adverse effects and ADME (absorption, distribution, metabolism, and excretion) profiles of stevioside in humans. The safety of whole-leaf or crude Stevia extracts for human consumption is still debated, highlighting the necessity for additional studies to understand the metabolic pathways of steviol glycosides and to assess their potential genotoxic risks.

Author's contribution

Samra Munir conceptualized and wrote the manuscript and other authors drafted, and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Competing Interest

The authors declared no conflict of interest.

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7. References

Ahmad, J., Khan, I., Johnson, S. K., Alam, I., and Din, Z. U. (2018). Effect of incorporating stevia and moringa in cookies on postprandial glycemia, appetite, palatability, and gastrointestinal well-being. Journal of the American College of Nutrition, 37(2), 133-139.

Anker, C. C. B., Rafiq, S., and Jeppesen, P. B. (2019). Effect of steviol glycosides on human health with emphasis on type 2 diabetic biomarkers: A systematic review and meta-analysis of randomized controlled trials. Nutrients, 11(9), 1965-1985.

Anton, S. D., Martin, C. K., Han, H., Coulon, S., Cefalu, W. T., Geiselman, P., and Williamson, D. A. (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite, 55(1), 37-43.

Ashwell, M. (2015). Stevia, nature's zero-calorie sustainable sweetener: A new player in the fight against obesity. Nutrition Today, 50(3), 129-134.

Assaei, R., Mokarram, P., Dastghaib, S., Darbandi, S., Darbandi, M., Zal, F., and Omrani, G. H. R. (2016). Hypoglycemic effect of aquatic extract of Stevia in the pancreas of diabetic rats: PPARγ-dependent regulation or antioxidant potential. Avicenna Journal of Medical Biotechnology, 8(2), 65-74.

Babu, A. K., Kumaresan, G., Raj, V. A. A., and Velraj, R. (2018). Review of leaf drying: Mechanism and influencing parameters, drying methods, nutrient preservation, and mathematical models. Renewable and Sustainable Energy Reviews, 90(7), 536-556.

Barriocanal, L. A., Palacios, M., Benitez, G., Benitez, S., Jimenez, J. T., Jimenez, N., and Rojas, V. (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and Type 1 and Type 2 diabetics. Regulatory Toxicology and Pharmacology, 51(1), 37-41.

Basit, A., Askari, S., Zafar, J., Riaz, M., Fawwad, A., and Members, N. D. S. P. (2021). NDSP 06: Prevalence and risk factors for obesity in urban and rural areas of Pakistan: A study from second National Diabetes Survey of Pakistan (NDSP), 2016–2017. Obesity Research and Clinical Practice, 15(1), 19-25.

Befa, A., Gebre, A., and Bekele, T. (2020). Evaluation of dried stevia (*Stevia rebaudiana* bertoni) leaf and its infusion nutritional profile. Medicine Aromatic Plants (Los Angeles), 9(360), 2167-412.

Benford, D., Hill, M. F., Schlatter, J., and DiNovi, M. (2009). Steviol glycosides (addendum). Safety Evaluation of Certain Food Additives, 60 (1), 183-219.

Blaauboer, B. J., Boobis, A. R., Bradford, B., Cockburn, A., Constable, A., Daneshian, M., and Schuermans, J. (2016). Considering new methodologies in strategies for safety assessment of foods and food ingredients. Food and Chemical Toxicology, 91(5), 19-35.

Carakostas, M. C., Curry, L. L., Boileau, A. C., and Brusick, D. J. (2008). Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food and Chemical Toxicology, 46(7), 1-10.

Carrera-Lanestosa, A., Moguel-Ordónez, Y., and Segura-Campos, M. (2017). *Stevia rebaudiana* Bertoni: a natural alternative for treating diseases associated with metabolic syndrome. Journal of Medicinal Food, 20(10), 933-943.

Chan, P., Tomlinson, B., Chen, Y. J., Liu, J. C., Hsieh, M. H., and Cheng, J. T. (2000). A double-blind placebo-a controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. British Journal of Clinical Pharmacology, 50(3), 215-220.

Chatsudthipong, V., and Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology and Therapeutics, 121(1), 41-54.

Chatterjee, S., Khunti, K., and Davies, M. J. (2017). Type 2 diabetes. The Lancet, 389(10085), 2239-2251.

Chaturvedula, V. S. P., and Prakash, I. (2011). Structures of the novel diterpene glycosides from *Stevia rebaudiana*.
Carbohydrate Research, 346(8), 1057-1060.

Chen, J., Xia, Y., Sui, X., Peng, Q., Zhang, T., Li, J., and Zhang, J. (2018). Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. Oncotarget, 9(41), 26299-26308.

Chen, T. H., Chen, S. C., Chan, P., Chu, Y. L., Yang, H. Y., and Cheng, J. T. (2005). Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. Planta Medica, 71(2), 108-113.

Colberg, S. R., Sigal, R. J., Yardley, J. E., Riddell, M. C., Dunstan, D. W., Dempsey, P. C., and Tate, D. F. (2016). Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. Diabetes Care, 39(11), 2065.

Conklin, A. I., Monsivais, P., Khaw, K. T., Wareham, N. J., and Forouhi, N. G. (2016). Dietary diversity, diet cost, and incidence of type 2 diabetes in the United Kingdom: a prospective cohort study. Public Library of Science Medicine, 13(7), 1002085-1002101.

Curi, R., Alvarez, M., Bazotte, R. B., Botion, L. M., Godoy, J. L., and Bracht, A. (1986). Effect of *Stevia rebaudiana* on glucose tolerance in normal adult humans. Brazilian Journal of Medical and Biological Research, 19(6), 771-774.

Das, S., Das, A. K., Murphy, R. A., Punwani, I. C., Nasution, M. P., and Kinghorn, A. D. (1992). Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. Caries Research, 26(5), 363-366.

Davies, M. J., D'Alessio, D. A., Fradkin, J., Kernan, W. N., Mathieu, C., Mingrone, G., ... and Buse, J. B. (2018). Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care, 41(12), 2669-2701.

De, S., Mondal, S., and Banerjee, S. (2013). Stevioside: technology, applications and health. John Wiley and Sons.

11(1) 1-18.

Durán Agüero, S., Vásquez Leiva, A., Morales Illanes, G., Schifferli Castro, I., Sanhueza Espinoza, C., Encina Vega, C., and Mena Bolvaran, F. (2015). Consumo de stevia en estudiantes universitarios chilenos y su asociación con el estado nutricional. Nutrición Hospitalaria, 32(1), 362-366.

Eckel, R. H., Kahn, S. E., Ferrannini, E., Goldfine, A. B., Nathan, D. M., Schwartz, M. W., and Smith, S. R. (2011). Obesity and type 2 diabetes: what can be unified and what needs to be individualized? The Journal of Clinical Endocrinology and Metabolism, 96(6), 1654-1663.

EFSA Panel on Food Additives and Flavourings (FAF), Younes, M., Aquilina, G., Engel, K. H., Fowler, P., Frutos Fernandez, M. J., and Castle, L. (2020). Safety of a proposed amendment of the specifications for steviol glycosides (E 960) as a food additive: To expand the list of steviol glycosides to all those identified in the leaves of *Stevia rebaudiana* Bertoni. European Food Safety Authority Journal, 18(4), 6106-6138.

EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS). (2010). Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive. European Food Safety Authority Journal, 8(4), 1537-1620.

Farhat, G., Berset, V., and Moore, L. (2019). Effects of stevia extract on postprandial glucose response, satiety and energy intake: a three-arm crossover trial. Nutrients, 11(12), 30-36.

Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., and Pietta, P. (2003). Metabolism of stevioside and rebaudioside A from *Stevia rebaudiana* extracts by human microflora. Journal of Agricultural and Food Chemistry, 51(22), 6618-6622.

Gerwig, G. J., Te Poele, E. M., Dijkhuizen, L., and Kamerling, J. P. (2016). Stevia glycosides: chemical and enzymatic modifications of their carbohydrate moieties to improve the sweet-tasting quality. Advances in Carbohydrate Chemistry and Biochemistry, 73(16), 51-62.

Goyal, S. K., Samsher, N., and Goyal, R. K. (2010). Stevia (*Stevia rebaudiana*) a bio-sweetener: A review. International Journal of Food Sciences and Nutrition, 61(1), 1-10.

Gregersen, S., Jeppesen, P. B., Holst, J. J., and Hermansen, K. (2004). Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism, 53(1), 73-76.

Gupta, E., Purwar, S., Sundaram, S., and Rai, G. K. (2013). Nutritional and therapeutic values of *Stevia rebaudiana*: A review. Journal of Medicinal Plants Research, 7(46), 3343-3353.

Gupta, E., Purwar, S., Singh, A., Sundaram, S., Rai, G. K., and Sundaram, S. (2015). Evaluation of nutritional, anti-nutritional and bioactive compounds in juice and powder of *Stevia rebaudiana*. Indian Journal of Natural Sciences, 5(28), 3308-3317.

Hsieh, M. H., Chan, P., Sue, Y. M., Liu, J. C., Liang, T. H., Huang, T. Y., and Chen, Y. J. (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study. Clinical Therapeutics, 25(11), 2797-2808.

Ibrahim, M., Tuomilehto, J., Aschner, P., Beseler, L., Cahn, A., Eckel, R. H., and Umpierrez, G. E. (2018). Global status of diabetes prevention and prospects for action: a consensus statement. Diabetes/Metabolism Research and Reviews, 34(6), 3021-3056.

Ibrahim, N. A., El-Gengaihi, S., Motawe, H., and Riad, S. A. (2007). Phytochemical and biological investigation of *Stevia rebaudiana* Bertoni; 1-labdane-type diterpene. European Food Research and Technology, 224(1), 483-488.

Jehan, A., and Ashoush, I. S. (2008). Antihyperglycemic effects of stevioside on diabetic rats. Pakistan Journal of Biotechnology, 5(2), 21-26.

Jeppesen, P. B., Gregersen, S., Rolfsen, S. E. D., Jepsen, M., Colombo, M., Agger, A., and Hermansen, K. (2003). Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism, 52(3), 372-378.

Kedik, S. A., Yartsev, E. I., and Stanishevskaya, I. E. (2009). Antiviral activity of dried extract of Stevia. Pharmaceutical Chemistry Journal, 43(4), 19-20.

Khan, M. K., Asif, M. N., Ahmad, M. H., Imran, M., Arshad, M. S., Hassan, S., ... and Muhammad, N. (2019). Ultrasound-assisted optimal development and characterization of stevia-sweetened functional beverage. Journal of Food Quality, 19(1), 1-6.

Khare, N., and Chandra, S. (2019). Stevioside mediated chemosensitization studies and cytotoxicity assay on breast cancer cell lines MDA-MB-231 and SKBR3. Saudi Journal of Biological Sciences, 26(7), 1596-1601.

Khiraoui, A., and Guedira, T. (2018). Effect of *Stevia rebaudiana*, sucrose and aspartame on human health: A comprehensive. Journal of Medicinal Plants Research, 6(1), 102-108.

Lambert, EV., and Bull, F. (2014). Public health recommendations for physical activity in the prevention of type 2 diabetes mellitus. Journal of Physical Activity and Health, 60(1), 130-40.

Lee, C. N., Wong, K. L., Liu, J. C., Chen, Y. J., Cheng, J. T., and Chan, P. (2001). Inhibitory effect of stevioside on calcium influx to produce antihypertension. Planta Medica, 67(9), 796-799.

Lemieux, I. (2020). Reversing type 2 diabetes: the time for lifestyle medicine has come! Nutrients, 12(7), 1974-1978.

Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., and Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chemistry, 132(3), 1121-1132.

Lestari, K., Ridho, A., Nurcayani, N., Ramadhania, Z. M., and Barliana, M. I. (2019). *Stevia rebaudiana* Bertoni leaves extract as a nutraceutical with hypoglycemic activity in diabetic rats. The Indonesian Biomedical Journal, 11(2), 182-187.

Li, G., Han, L., Wang, Y., Zhao, Y., Li, Y., Fu, J., and Willi, S. M. (2018). Evaluation of ADA HbA1c criteria in the diagnosis of pre-diabetes and diabetes in a population of Chinese adolescents and young adults at high risk for diabetes: a cross-sectional study. Biomedical Journal Open, 8(8), 20665-20674.

Li, T., Zhao, M., Raza, A., Guo, J., He, T., Zou, T., and Song, H. (2020). The effect of taste and taste perception on satiation/satiety: a review. Food and Function, 11(4), 2838-2847.

Lipska, K. J., Krumholz, H., Soones, T., and Lee, S. J. (2016). Polypharmacy in the aging patient: a review of glycemic control in older adults with type 2 diabetes. Journal of the American Medical Association, 315(10), 1034-1045.

López, V., Pérez, S., Vinuesa, A., Zorzetto, C., and Abian, O. (2016). *Stevia rebaudiana* ethanolic extract exerts better antioxidant properties and antiproliferative effects in tumor cells than its diterpene glycoside stevioside. Food and Function, 7(4), 2107-2113.

Madan, S. M., Ahmad, S. A., Singh, G. N., Kohli, K. K., Kumar, Y. K., Singh, R. S., and Garg, M. (2010). Stevia rebaudiana (Bert.) Bertoni-a review. Indian Journal of Natural Products and Resources, 1(3), 267-286.

Magnuson, B. A., Carakostas, M. C., Moore, N. H., Poulos, S. P., and Renwick, A. G. (2016). Biological fate of low-calorie sweeteners. Nutrition Reviews, 74(11), 670-689.

Maki, K. C., Curry, L. L., McKenney, J. M., Farmer, M. V., Reeves, M. S., Dicklin, M. R., and Zinman, B. (2009). Glycemic and blood pressure responses to acute doses of rebaudioside a, a steviol glycoside, in subjects with normal glucose tolerance or type 2 diabetes mellitus. The Federation of American Societies for Experimental Biology Journal, 23(1), 351-356.

Mandal, B., and Madan, S. (2013). Preliminary phytochemical screening and evaluation of free radical scavenging activity of *Stevia rebaudiana* Bertoni from different geographical sources. Journal of Pharmacognosy and Phytochemistry, 2(1), 14-19.

Manjunatha, S. N., and Jayashree, S. (2019). Perceived challenges in diabetic care: A cross sectional study among in-patients of a tertiary care hospital. Indian Journal of Forensic and Community Medicine, 6(4), 208-210.

Mathur, S., Bulchandani, N., Parihar, S., and Shekhawat, G. S. (2017). Critical review on steviol glycosides: Pharmacological, toxicological, and therapeutic aspects of high potency zero caloric sweetener. International Journal of Pharmacology, 13(7), 916-928.

McCracken, E., Monaghan, M., and Sreenivasan, S. (2018). Pathophysiology of the metabolic syndrome. Clinics in Dermatology, 36(1), 14-20.

Meesschaert, B., Moons, N., Steurs, G., Monballiu, A., Amery, R., Jooken, E., and Geuns, J. (2021). Degradation of steviol glycosides via steviol and Monicanone by soil microorganisms and UASB effluent. Journal of Environmental Chemical Engineering, 9(6), 106342-106353.

McGown, C., Birerdinc, A., and Younossi, Z. M. (2014). Adipose tissue is an endocrine organ. Clinics in Liver Disease, 18(1), 41-58.

Mohan, K., and Robert, J. (2009). Hepatoprotective effects of *Stevia rebaudiana* Bertoni leaf extract in CCl4-induced liver injury in albino rats. Medicinal and Aromatic Plant Science Biotechnology, 3(1), 59-61.

Nguyen, M., Jarvis, S. E., Tinajero, M. G., Yu, J., Chiavaroli, L., Mejia, S. B., and Malik, V. S. (2023). Sugar-sweetened beverage consumption and weight gain in children and adults: A systematic review and meta-analysis of prospective cohort studies and randomized controlled trials. The American Journal of Clinical Nutrition, 117(1), 160-174.

Olson, D. E., Rhee, M. K., Herrick, K., Ziemer, D. C., Twombly, J. G., and Phillips, L. S. (2010). Screening for diabetes and pre-diabetes with proposed A1C-based diagnostic criteria. Diabetes Care, 33(10), 2184-2189.

Overbeek, J. A., Heintjes, E. M., Prieto-Alhambra, D., Blin, P., Lassalle, R., Hall, G. C., and Herings, R. M. (2017). Type 2 diabetes mellitus treatment patterns across Europe: a population-based multi-database study. Clinical Therapeutics, 39(4), 759-770.

Pang, M. D., Goossens, G. H., and Blaak, E. E. (2021). The impact of artificial sweeteners on body weight control and glucose homeostasis. Frontiers in Nutrition, 7(1), 333.

Pól, J., Hohnová, B., and Hyötyläinen, T. (2007). Characterisation of *Stevia rebaudiana* by comprehensive two--dimensional liquid chromatography time-of-flight mass spectrometry. Journal of Chromatography A, 1150(2), 85-92.

Prakash, I., and Chaturvedula, V. S. P. (2018). Steviol glycosides: natural noncaloric sweeteners. Reference Series in Phytochemistry. Springer, 18(5), 101-128.

Prakash, I., DuBois, G. E., Clos, J. F., Wilkens, K. L., and Fosdick, L. E. (2008). Development of rebiana, a natural, non-caloric sweetener. Food and Chemical Toxicology, 46(7), 75-82.

Purkayastha, S., Bhusari, S., Pugh, G., Teng, X., Kwok, D., and Tarka, S. M. (2015). In vitro metabolism of rebaudioside E under anaerobic conditions: comparison with rebaudioside A. Regulatory Toxicology and Pharmacology, 72(3), 646-657.

Qaid, M. M., and Abdelrahman, M. M. (2016). Role of insulin and other related hormones in energy metabolism-A review. Cogent Food and Agriculture, 2(1), 1267691-1267709.

Qazi, S., Siddiqui, I. A., Saeed, M., Perveen, K., Baqa, K., and Fawwad, A. (2023). Association of serum level of chemerin with visceral fat obesity in type 2 diabetic patients. International Journal of Diabetes in Developing Countries, 43(2), 298-303.

Rashad, N. M., Abdelsamad, M. A., Amer, A. M., Sitohy, M. Z., and Mousa, M. M. (2019). The impact of stevioside supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A controlled clinical trial. The Egyptian Journal of Internal Medicine, 31(1), 22-30.

Rashid, Z., Ahngar, T. A., Nazir, A., Dar, Z. A., Khuroo, N. S., Majeed, S., and Jan, S. (2021). Research Technology of Stevia, 10 (3), 1-14.

Ray, J., Kumar, S., Laor, D., Shereen, N., Nwamaghinna, F., Thomson, A., and McFarlane, S. I. (2020). Effects of *Stevia rebaudiana* on glucose homeostasis, blood pressure, and inflammation: a critical review of past and current research evidence. International Journal of Clinical Research and Trials, 5(3), 142-153.

Ren, H. P., Yin, X. Y., Yu, H. Y., and Xiao, H. F. (2017). Stevioside-induced cytotoxicity in colon cancer cells via reactive oxygen species and mitogen-activated protein kinase signaling pathways-mediated apoptosis. Oncology Letters, 13(4), 2337-2343.

Ritu, M., and Nandini, J. (2016). The nutritional composition of *Stevia rebaudiana*, a sweet herb, and its hypoglycaemic and hypolipidaemic effect on patients with non-insulin dependent diabetes mellitus. Journal of the Science of Food and Agriculture, 96(12), 4231-4234.

Roglic, G. (2016). WHO Global report on diabetes: A summary. International Journal of Noncommunicable Diseases, 1(1), 3-8.

Rojas, E., Bermúdez, V., Motlaghzadeh, Y., Mathew, J., Fidilio, E., Faria, J., and Kuzmar, I. (2018). Stevia rebaudiana Bertoni and its effects in human disease: emphasizing its role in inflammation, atherosclerosis, and metabolic syndrome. Current Nutrition Reports, 7(7), 161-170.

Rubenstrunk, A., Hanf, R., Hum, D. W., Fruchart, J. C., and Staels, B. (2007). Safety issues and prospects for the future generations of PPAR modulators. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1771(8), 1065-1081.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., and IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Research and Clinical Practice, 157(19), 107843-107852.

Sami, W., Ansari, T., Butt, N. S., and Ab Hamid, M. R. (2017). Effect of diet on type 2 diabetes mellitus: A review. International Journal of Health Sciences, 11(2), 65.

Samsudin, A., and Aziz, I. A. (2013). Drying of stevia leaves using laboratory and pilot scale dryers. Journal of Tropical Agriculture and Food Science, 41(1), 137-147.

Samuel, P., Ayoob, K. T., Magnuson, B. A., Wölwer-Rieck, U., Jeppesen, P. B., Rogers, P. J., and Mathews, R.

(2018). Stevia leaf to stevia sweetener: exploring its science, benefits, and future potential. The Journal of Nutrition, 148(7), 1186-1205.

Sathishkumar Jayaraman, S. J., Manoharan, M. S., and Seethalakshmi Illanchezian, S. I. (2008). In-vitro antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Tropical Journal of Pharmaceutical Research, 7(4), 1143-1149.

Scheen, A. J. (2015). Pharmacodynamics, efficacy and safety of sodium–glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. Drugs, 75(15), 33-59.

Schwartz, M. W., Seeley, R. J., Zeltser, L. M., Drewnowski, A., Ravussin, E., Redman, L. M., and Leibel, R. L. (2017). Obesity pathogenesis: an endocrine society scientific statement. Endocrine Reviews, 38(4), 267-296.

Shahid, S., Akhter, Z., Sukaina, M., Sohail, F., Nasir, F., Sukaina II, M., and Sohail IV, F. (2021). Association of diabetes with lower back pain: a narrative review. Cureus, 13(6).

Shahu, R., Jobby, R., Patil, S., Bhori, M., Tungare, K., and Jha, P. (2022). Phytochemical content and antioxidant the activity of different varieties of *Stevia rebaudiana*. Horticulture, Environment, and Biotechnology, 63(6), 935-948.

Sharma, N., Mogra, R., and Upadhyay, B. (2009). Effect of stevia extract intervention on lipid profile. Studies on Ethno-Medicine, 3(2), 137-140.

Shawahna, R., Samaro, S., and Ahmad, Z. (2021). Knowledge, attitude, and practice of patients with type 2 diabetes mellitus about their disease: a cross-sectional study among Palestinians of the West Bank. BMC Public Health, 21(472), 1-13.

Siddiqui, M., Hameed, R., Nadeem, M., Mohammad, T., Simbak, N., Latif, A., and Baig, A. (2018). Obesity in Pakistan; current and future perceptions. J Curr Trends Biomed Eng Biosci, 17(2), 555958.

Silva, G. E. C. D., Assef, A. H., Albino, C. C., Ferri, L. D. A. F., Tasin, G., Takahashi, M. H., and Bazotte, R. B. (2006). Investigation of the tolerability of oral stevioside in Brazilian hyperlipidemic patients. Brazilian Archives of Biology and Technology, 49(4), 583-587.

Singh, S. D., and Rao, G. P. (2005). Stevia: The herbal sugar of the 21st century. Sugar Technology, 7(1), 17-24.

Soares, A. F., Honorio, A. R., de Lima, D. C. N., and Tribst, A. A. (2021). Sweet processed foods in Brazil: use of sugar and sweeteners, inclusion of sugar claims, and impact on nutritional profile. International Journal of Food Science and Technology, 56(9), 4428-4433.

Sunanda, S., and Veena, G. (2014). Antidiabetic, antidyslipidymic, and antioxidative potential of methanolic root extract of *Stevia rebaudiana* (Bertoni) on alloxan induced diabetic mice. World Journal of Pharmacy and Pharmaceutical Sciences, 3(7), 1859-1872.

Takahashi, K., Iwata, Y., Mori, S., and Shigeta, S. (1998). In-vitro anti-HIV activity of extract from Stevia rebaudiana. Antiviral Res, 37(1), 59-62.

Takahashi, K., Matsuda, M., Ohashi, K., Taniguchi, K., Nakagomi, O., Abe, Y., and Shigeta, S. (2001). Analysis of the anti-rotavirus activity of extract from *Stevia rebaudiana*. Antiviral Research, 49(1), 15-24.

Talevi, A. (2022). Potential medicinal effects and applications of stevia constituents. Phytochemistry Reviews, 21(1), 161-178.

Ulbricht, C., Isaac, R., Milkin, T., A Poole, E., Rusie, E., M Grimes Serrano, J., and Woods, J. (2010). An evidence-based systematic review of stevia by the Natural Standard Research Collaboration. Cardiovascular and Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular and Hematological Agents), 8(2), 113-127.

Van Gaal, L., and Scheen, A. (2015). Weight management in type 2 diabetes: current and emerging approaches to treatment. Diabetes care, 38(6), 1161-1172.

Verma, S., and Hussain, M. E. (2017). Obesity and diabetes: an update. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 11(1), 73-79.

Wang, M., Lu, J., Li, J., Qi, H., Wang, Y., and Zhang, H. (2014). Steviol glucuronidation and its potential interaction with UDP-glucuronosyltransferase 2B7 substrates. Food and Chemical Toxicology, 64(1), 135-143.

Wang, J., Zhao, H., Wang, Y., Lau, H., Zhou, W., Chen, C., and Tan, S. (2020). A review of stevia as a potential healthcare product: Up-to-date functional characteristics, administrative standards, and engineering techniques. Trends in Food Science and Technology, 103(10), 264-281.

Wang, L., and Wu, W. (2019). Angiotensin-converting enzyme inhibiting ability of ethanol extracts, steviol

glycosides and protein hydrolysates from stevia leaves. Food and Function, 10(12), 7967-7972.
Wang, Y., Luo, X., Chen, L., Mustapha, A. T., Yu, X., Zhou, C., and Okonkwo, C. E. (2023). Natural and low-caloric rebaudioside A as a substitute for dietary sugars: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety, 22(1), 615-642.
Wilding, J. (2014). The importance of weight management in type 2 diabetes mellitus. International Journal of Clinical Practice, 68(6), 682-691.
World Health Organization. (2003). Diet, nutrition, and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation (Vol. 916). World Health Organization.
Yadav, K., and Guleria, P., (2012). Steviol glycosides from Stevia: Biosynthesis pathway review and their application in foods and medicine. Critical Reviews in Food Science and Nutrition, 52(11), 988-998.