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The functional and health-promoting properties of *Stevia rebaudiana* Bertoni and its glycosides with special focus on the antidiabetic potential – A review

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ABSTRACT

Stevia and its glycosides are becoming more popular both in the world of science and in the food industry. This review discusses the functional properties of *Stevia*, which is mainly used as an alternative sweetener. The results of research revealed significant health-promoting properties of *Stevia* and steviol glycosides applied at certain doses and under specific conditions. *Stevia* preparations exhibit anti-inflammatory, oral health-promoting, antihypertensive, and chemopreventive effects. Moreover, they help to regulate glycaemia, inter alia, by affecting glucose uptake, improving insulin secretion or increasing the concentration of glucose transporters. Most of the results concerning the antidiabetic properties of *Stevia* come from *in vitro* and *in vivo* studies. So far there have been only a few clinical trials conducted on humans. As the mechanisms underlying these effects have not been fully understood, they require further investigation. This article presents the state of the art concerning this issue.

1. Introduction

Stevia rebaudiana Bertoni (USDA 'Stevia rebaudiana (Bertoni)'), which is commonly known as candyleaf, is a species of *Stevia*. The plant is famous for its great sweetness due to the content of steviol glycosides, which are classified as food additives under the number E960 in the European Union. The full name of the plant refers to Moisés Santiago Bertoni, who was the first to describe this specimen officially in Paraguay in 1899 (Bertoni, 1899)). In 1970 *Stevia* was commercially introduced to the market in Japan as a sweetener (Ashok, Singh, Dhyani, & Ahuja, 2011). Today steviol glycosides are used for food production in the United States, Canada, Brazil, South Africa and in the European Union. The availability and legal status of *Stevia* is uncertain in many places around the world, but this situation is changing gradually and the use of *Stevia* extracts is more and more common. For example, they can be found in chocolate, yoghurts, ketchups, canned vegetables, jams, ice cream, chewing gums, beverages and table-top sweeteners.

The highest content of steviol glycosides is concentrated in leaves. The substances can be acquired in various ways. Originally, various solvents were used for this process, such as ethanol or methanol, by means of which steviol glycosides were extracted from dried and crushed plants. Methods based on adsorption chromatography, ion exchange, and selective precipitation of specific steviol glycosides have been developed (Brandle, Starratt, & Gijzen, 1998). Commercially, these compounds are extracted in several steps. First, dried and shredded leaves are enzymatically hydrolysed. During the process the structures of the raw material cell walls are broken. After steeping in hot water or an organic solvent such as ethanol the extract contains all soluble components of the plant. It is essential to remove all leaf residue and other undesirable compounds in the purification process, for instance, by changing pH or adding inorganic salts. Finally, during the filtration process the precipitate is separated from the solution. Steviol glycoside powder is obtained by spray drying. In order to isolate the highest purity product it is necessary to apply techniques based on multiple steeping in alcohol, membrane ultrafiltration and crystallisation (Kolanowski, 2013).

Steviol glycosides are generally considered to be safe. They are not hydrolysed enzymatically or absorbed in the upper gastrointestinal tract (Geuns, Buyse, Vankeirsbilck, & Temme, 2007). They are broken down only by bacteria living in the colon. The results of studies are not always convergent, but most of them prove the safety of consumption of these sweeteners. The Food and Agriculture Organisation of the United

Abbreviations: ADI, acceptable daily intake; Akt, protein kinases B; AMPK, 5′AMP-activated protein kinase; CFTR, cystic fibrosis transmembrane conductance regulator; FAO, The Food and Agriculture Organisation of the United Nations; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; HPLC, high performance liquid chromatography; PI3K, phosphoinositide 3-kinases; TRPM5, transient receptor potential cation channel subfamily melastatin member 5; WHO, World Health Organisation; WT, wild type mice

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Nations (FAO) has set the acceptable daily intake (ADI) of steviol glycosides at 0–4 mg/kg of body weight (Perrier, Mihalov, & Carlson, 2018). For safety control the level of steviol glycosides in food is usually estimated by means of high performance liquid chromatography (HPLC). It is a reference method established by the FAO (FAO/WHO, 2017), and its effectiveness was confirmed by latest research (Fayaz, Sharma, & Bimlesh, 2018).

The interest of the national food industry in steviol glycosides is growing mainly because they are much sweeter than sucrose. They have great industrial potential but their wide use is doubtful. All sweeteners are often compared with table sugar. One of the biggest problems is that steviol glycosides do not have many desirable properties of sucrose. For instance, *Stevia* sweeteners do not form a texture and do not have positive influence on the colour of the product. Therefore, steviol glycosides cannot replace sucrose at a 1:1 ratio (Karp, Kurek, & Wierzbicka, 2016). They are treated mainly as a sugar substitute in the food industry, but they are not very similar to sucrose. Nevertheless, steviol glycosides have important advantages. The zero energy value and great health safety makes them superior to table sugar. What is more, studies showed that the consumption of *Stevia rebaudiana* Bertoni preparations may be beneficial for health. Steviol glycosides do not accumulate in the body and have numerous health-promoting properties, as they exhibit antioxidative, antibacterial, antifungal, anticaries, hypotensive or hypoglycaemic effects (Gupta, Purwar, Sundaram, & Rai, 2013). Many scientists are conducting extensive research on the influence of steviol glycosides on health. There are promising reports that these substances may help to prevent and support the treatment of type 2 diabetes, which is a dangerous and increasingly common disease. According to the World Health Organisation (WHO), the number of diabetic patients around the world increased from 108 million in 1980 to 422 million in 2014. It means that almost 9% of the world population suffers from diabetes mellitus (WHO, 2017). It is extremely important to search for new ways of fighting diabetes mellitus because current treatment strategies still have many disadvantages (various efficacy, side effects, high costs). There are serious consequences of diabetes, which is a risk factor for many other life-threatening diseases and incidents.

Stevioside and rebaudioside A were the first glycosides whose positive effect was appreciated and relatively well characterised due to their high content in the plant. There are differences in details concerning the content of specific steviol glycosides in the plant. According to reference publications, stevioside (4–13%) is the most common of all steviol glycosides. It is followed by rebaudioside A (2–4%), rebaudioside C (1–2%) and dulcoside A (0.4–0.7%). However, these data were published in 1984 (Makapugay, Nanayakkara, & Kinghorn, 1984). According to recent reports, there is high content of rubusoside and a much lower amount of dulcoside A (Fig. 1) (Bender, Graziano, & Zimmermann, 2015; Brandle et al., 1998; Makapugay et al., 1984). Nevertheless, these values may vary according to the growing conditions and harvest time (Hanson, 2016).

2. Functional properties

2.1. Taste and sweetness of Stevia

The sweetness intensity of *Stevia* is the subject of many studies. High-quality *Stevia* leaves are about 30 times sweeter than table sugar (Savita et al., 2004). Studies provide various data on the sweetening power of steviol glycosides. They are up to several hundred times sweeter than sucrose, depending on specific compounds (Belloir, Neiers, & Briand, 2017; Prakash, Dubois, Clos, Wilkens, & Fosdick, 2008) (Fig. 2). Differences in the data concerning the sweetness of *Stevia* may result from the purity of the substance or differences in the specificity of taste receptors. Moreover, depending on the concentration, the taste of steviol glycosides is manifested differently than the taste of sucrose (Cardello, Da Silva, & Damasio, 1999). Studies have shown that as the concentration of stevioside in a solution increases, its sweetness does not grow as efficiently as the sweetness of a solution with sucrose. It means that a double concentration of sucrose causes double sweetness of the solution, but it is not the case with steviol glycosides. The effect is not so efficient and a greater dose of the agent is necessary to equalise the level of sweetness. Therefore, it is difficult to estimate the sweetening power of *Stevia* precisely.

Stevioside and rebaudioside A are the most common steviol glycosides in the food industry. The latter is gaining popularity not only because of its sweetening power but also because it is largely free from the bitter aftertaste. By contrast, rebaudioside M not only has a slightly bitter aftertaste but also a liquorice one. Rubusoside, on the other hand, is the most bitter of all. According to the latest research, in contact with the hT2R4 and hT2R14 bitter taste receptors steviol glycosides stimulate them and cause a bitter aftertaste (Acevedo, Gonzalez-Nilo, & Agosin, 2016; Prakash, Markosyan, & Bunders, 2014). The specific perception of sweetness delivered by steviol glycosides is an important issue as it is distinctly longer than that of sucrose. It is essential to remember it when designing new products containing *Stevia* (Savita et al., 2004).

2.2. Overall stability of Stevia

The resistance of steviol glycosides to high temperature, variable pH and long-term exposure to sunlight was proved as early as the 1980s (Brandle et al., 1998). This study along with the current use of steviol glycosides in the food industry suggests that *Stevia* preparations have a great potential as a high-intensity, non-caloric sweetener.

More recent research shows that the stability of steviol glycosides

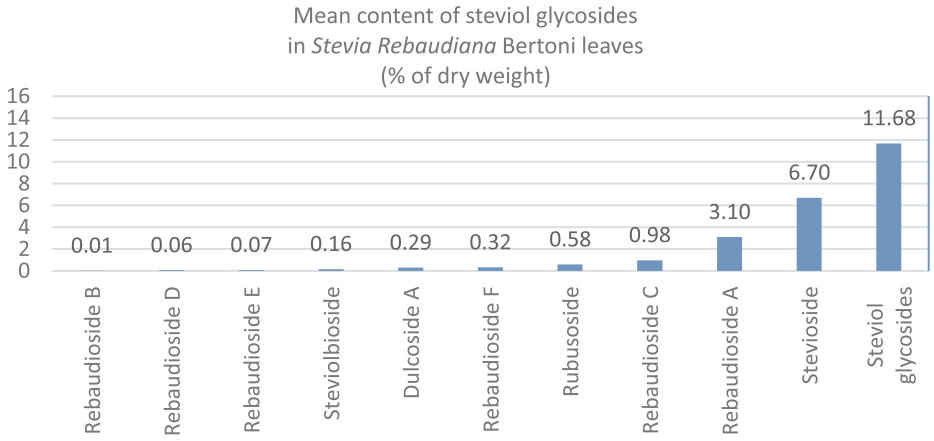

Fig. 1. The content of steviol glycosides in *Stevia rebaudiana* leaves (% of dry weight). (Bender et al., 2015; Brandle et al., 1998; Makapugay et al., 1984).

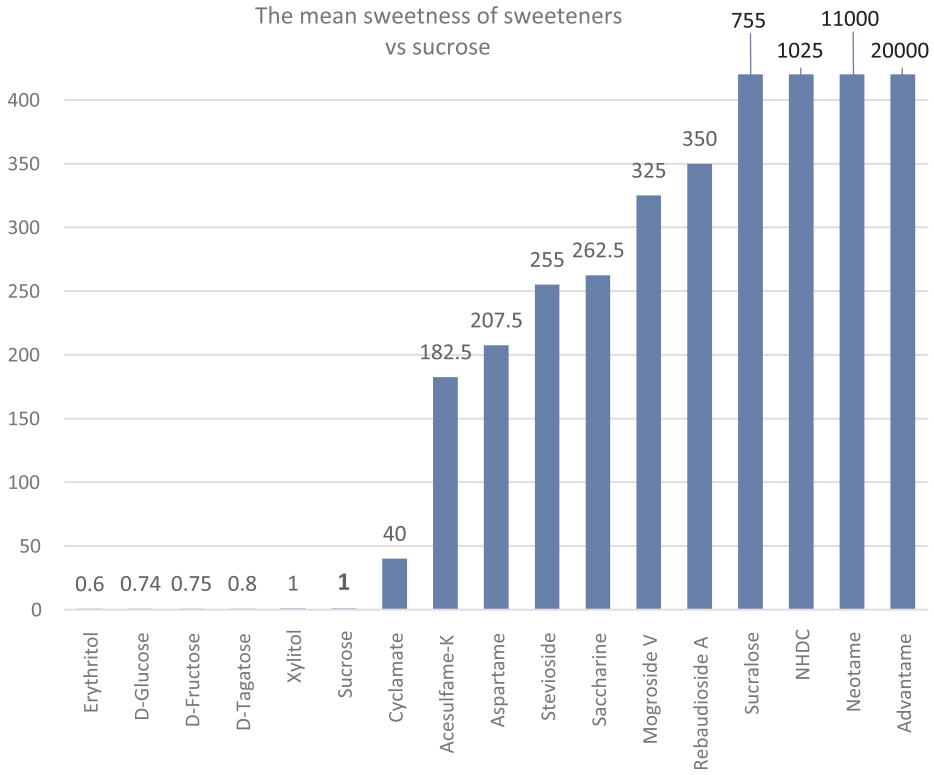

Fig. 2. The mean sweetness of sweeteners vs sucrose (Belloir et al., 2017; Prakash et al., 2008).

guarantees complete preservation of functional properties, including the sweet taste, at temperatures up to 120 °C for one hour (Kroyer, 2010). These compounds are highly resistant to a wide range of pH changes. Stevioside samples analysed after being subjected to a pH of 2–10 preserved their properties even at elevated temperature (80 °C). Only when pH was lower than 2, the compound became significantly degraded (Kroyer, 2010). The stability of rebaudioside A is well maintained within a pH range of 4–8. It deteriorates considerably when pH drops below 2 (Prakash et al., 2008).

2.3. Use of Stevia in food matrices

A study on the sweetness intensity of muffins baked with the addition of steviol glycosides showed that the product had a satisfactory flavour profile even when baked at a temperature of 180 °C for 20 min (Karp et al., 2016). On the other hand, the same study showed that when the amount of steviol glycosides increased, whereas the amount of sucrose decreased, the yield of cooking was poorer. What is more, the colour and browning index parameters deteriorated along with the declining baking performance. It resulted from the smaller amount of Maillard reaction products and the fact that steviol glycosides are not susceptible to caramelisation (Hamzah, Aluwi, & Sembok, 2013).

The study analysing the fate of stevioside and rebaudioside A in beverages exposed to sunlight showed hardly any degradation (Clos, DuBois, & Prakash, 2008). The authors presumed that the mild deterioration of the agents under analysis was probably caused by very low pH of beverages rather than by the exposure to sunlight.

Also, it is known that steviol glycosides are invulnerable to fermentation processes (Ruiz-Ruiz, Moguel-Ordoñez, Matus-Basto, & Segura-Campos, 2015). The fact that these compounds will not affect fermentation may be very important in food production, e.g. bakery production. The addition of steviol glycosides will only make finished products sweeter. Furthermore, unlike sucrose, they will not affect the colour of products during baking (Mishra, 2011).

The overall in-time stability of steviol glycosides in food products is mostly satisfactory and these compounds retain their functional properties relatively long. Jooken et al. (2012) reported that changes in the concentration of stevioside and rebaudioside A in a semi-skimmed soy beverage and fermented milk drink during 20-week storage at 6 and 20 °C were not significant. The storage of ice cream gave similar results. The content of steviol glycosides remained the same for 12 weeks (at −18 °C). Also, these compounds exhibited robust stability in skimmed and full-fat yoghurts for 34 days (at 6 °C). Stevioside and rebaudioside A were also highly effective as fruit jam sweeteners – they remained stable in spite of additional exposure to ambient light. Rebaudioside A remained stable even after exposure to high temperatures, for instance, during baking at 185 °C and storage for 4 weeks. Stevioside exposed to a temperature of 120 °C fully preserved its structure as well. There were minimal losses observed at 140 °C (Kroyer, 2010).

Nowicka and Wojdyło (2016) studied the effect of various sweeteners (sucrose, palm sugar, erythritol, xylitol, steviol glycosides and luo han guo) on the stability of phenolic compounds, the antioxidative activity and colour of sour cherry puree. The researchers observed that after storing the product containing steviol glycosides at 4 °C for 6 months the content of phenolic compounds decreased by 16%. There were better results observed in products with palm sugar and erythritol only. However, after 6 months at 30 °C the content of phenolic compounds decreased by 54% in the *Stevia* product, which was the largest decrease in comparison with the results of the other sweetening products. Nevertheless, the content of phenolic compounds in the product with steviol glycosides was very high at the beginning of the experiment. In comparison with the other sweetening products, the value of this parameter was relatively satisfactory. The antioxidative activity of all sweeteners dropped substantially after 6 months of storage. Among all products containing sweeteners the product with steviol glycosides exhibited the highest antioxidative activity at the beginning of the experiment and a relatively low decrease in this parameter after the storage period. In comparison with the standard product, the colour of the sour cherry puree deteriorated in almost all products with sweeteners.

3. Therapeutic and health-promoting properties of *Stevia* and its glycosides

There is a great number of documented reports concerning the potential therapeutic and health-promoting benefits of *Stevia* extracts and its pure compounds. Most studies were conducted on various *in vitro* and *in vivo* models, but studies on humans are still insufficient to formulate conclusions about applications. This review, due to limited volume, does not encompass all existing reports, thus focuses on selected results obtained in model studies, while human trials are discussed in the last paragraph.

3.1. Anti-inflammatory and immunomodulatory properties

Steviol glycosides have been reported to exhibit anti-inflammatory properties in various animal models (Wang et al., 2014). For example, a study on 30 6–8-week-old lactating mice (BALB/c) was conducted to examine whether stevioside could reduce inflammation. The researchers used *Staphylococcus aureus* to infect the animals' mammary glands and induce inflammation. The agent was administered daily by intraperitoneal injection at concentrations of 33, 100 and 300 mg/kg bw. The analysis of mammary gland tissues showed that the expression of TNF-α, IL-1β, and IL-6 in the infected mice treated with stevioside was suppressed in a dose-dependent manner. Stevioside inhibited the phosphorylation of IκBα, p65, p38, ERK, and JNK, and affected the inflammation by regulating the NF-κB and MAPK pathway. The agent also suppressed the expression of the TLR2 gene, which is responsible for the regulation of apoptosis and inflammation. The researchers concluded that **stevioside reduced the inflammation without undesirable effects** on the healthy animals.

Yuajit et al. (2014) examined the effect of steviol on polycystic kidney disease on a special breed of mice generated for the experiment – $Pkd1^{flox/flox}$:Pkhd1-Cre. The mice's genotype determined the tendency to develop cystic lesions in the kidneys. The animals were divided into 2 main groups: a DMSO-treated group and an experimental group treated with steviol or stevioside (dissolved in DMSO and distilled water). Steviol was administered by intraperitoneal injection at daily doses of 40 and 200 mg/kg bw, while stevioside was administered orally at daily doses of 500, 700 and 1000 mg/kg bw. The analysis of isolated kidneys showed that steviol (200 mg) had inhibited renal cystogenesis. The agent improved the cystic index and renal function and significantly decreased the cystic area, weight and size of kidneys. Stevioside (1000 mg) inhibited the cyst growth. The treatment decreased the kidney weight and cystic index. It was found that the mechanism underlying this renal-remedial effect was associated with reduced expression of the chloride channel of the cystic fibrosis transmembrane conductance regulator (CFTR), the inhibition of cell proliferation in kidneys was partly caused by affecting the mTOR/S6K signalling pathway, whereas the inhibition of the CFTR channel activity was associated with the activation of 5′AMP-activated protein kinase (AMPK). The study showed that **steviol could decrease cyst progression and improve the renal function**.

Casas-Grajales et al. (2019) conducted an *in vitro* and *in vivo* study to investigate the possible immunomodulatory effects of stevioside. They found that the agent (1 – 100 mM) was able to prevent the elevation of proinflammatory proteins (NF-κB, TNF-α, and IL-6) caused by lipopolysaccharide/ethanol exposure in hHSC/VL-17A cells. The *in vivo* trial on Wistar rats showed that stevioside (20 mg/kg, twice per day) prevented the diminution of glycogen caused by thioacetamide, dimished the elevation of proinflammatory cytokines (IL-17a, IL-1β, TNF-α, IL-6 and IL-10) in injured rats. This study proved that **stevioside exhibited immunomodulatory activity and** was able to **preserve liver functionality** under specific conditions.

3.2. Oral health-promoting properties

Stevia preparations have been reported to have some oral health-promoting properties. The compounds contained in this plant may be used as antiplaque agents because they form lighter biofilm and reduce bacterial adherence (Abdul Razak et al., 2017). For example, Vandana et al. (2017) conducted a six-month study on 108 female schoolchildren (12–15 years old) to examine the effectiveness of stevioside as a daily mouth rinse. The experiment showed that **stevioside was effective in the treatment and prevention of oral diseases**, as it reduced plaque and alleviated gingivitis.

The antiplaque effect of *Stevia* preparations was recently confirmed by Siraj, Pushpanjali, and Manoranjitha (2019) in a two-month trial on 22 participants (8 men and 14 women). The experiment showed that a 0.2% ***Stevia* leaf extract and *Stevia* mouth rinse product were bacteriostatic, stabilised** the plaque **pH and exhibited anti-caries activity**.

3.3. Antimicrobial properties

Herbal plants have gained attention as alternative medications because of their therapeutic properties against a wide range of infectious diseases caused by bacteria, fungi and viruses. *Stevia* preparations have been reported to have the potential to inhibit the activity of pathogenic bacteria, but it is not known which compound in *Stevia* leaves is responsible for this effect. Trials on the influence of *Stevia* preparations on the activity of pathogenic bacteria appeared to be promising. Sansano, Rivas, Pina-Pérez, Martinez, and Rodrigo (2017) studied the effect of infusion of *Stevia* leaves on the haemolytic capability of *L. monocytogenes*. The researchers found that at low temperature the activity of *L. monocytogenes* (measured with Listeriolysin O production) was low, regardless of the presence of the infusion. However, higher temperature caused a robust increase in the bacterial activity in the 0% matrix. Both the 1.25% and 2.5% ***Stevia* leaf infusion matrices reduced the production of Listeriolysin O significantly**. The activity of *L. monocytogenes* did not depend on the bacterial concentration.

Jayaraman, Manoharan, and Illanchezian (2008) investigated the antimicrobial activity of *Stevia rebaudiana* leaf extracts (100 mg/ml). The researchers found that all tested preparations (ethyl acetate, acetone, chloroform and water extracts) were effective against *Candida albicans* and *Epidermophyton* species. Acetone and ethyl acetate extracts were the most effective against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Vibrio cholerae, Aeromonas hydrophila and Escherichia coli*.

Abou-Arab and Abu-Salem (2010) confirmed the antibacterial and antifungal activity of *Stevia rebaudiana* extracts (100 mg/mL). The researchers observed that among all extracts (water, acetone, chloroform, methanol, ethyl acetate and hexane) methanol and acetone preparations were the most efficacious against *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus and Pseudomonas aeruginosa*. The activity of *Asperigillus parasiticus, Asperigillus ochraceus, Fusarium and Asperigillus flavus* was inhibited most by chloroform, methanol, acetone and ethyl acetate extracts. The research results proved the **significant antimicrobial potential of *Stevia* preparations**.

Steviol glycosides cannot be broken down by human digestive enzymes and they are not absorbed in the upper gastrointestinal tract. These compounds are utilised by intestinal microbiota (Gardana, Simonetti, Canzi, Zanchi, & Pietta, 2003), so hypothetically they may act as prebiotics. However, the results of experiments investigating the potential of stevioside and rebaudioside A to enhance the growth of probiotics were not promising (Deniņa, Semjonovs, Fomina, Treimane, & Linde, 2014; Kunová, Rada, Vidaillac, & Lisova, 2014). Strains of *Lactobacillus* and *Bifidobacterium* were not able to efficiently utilise these agents as a carbon source, so the bacterial growth was ultimately unsatisfactory and selective.

3.4. Cardiovascular properties

Antihypertensive properties of steviol glycosides have been observed both in animals and humans. For example, Chan et al. (1998) observed the **hypotensive effect of stevioside administered intravenously** (200 mg/kg bw.) to hypertensive rats. A later experiment conducted in the same laboratory confirmed this effect and indicated that the mechanism of action was based on Ca^{2+} flux inhibition (Lee et al., 2001). Hsieh et al. (2003) conducted a long-term study on humans to investigate the hypotensive properties of stevioside. A group of 168 Chinese men and women aged 20–75 years with mild hypertension was divided into two randomised groups: a group treated with 500 mg capsules of stevioside administered orally 3 times a day (82 subjects) and a group treated with a matching placebo (86 subjects). The treatment significantly decreased both the systolic and diastolic blood pressure. The effect was achieved after one week of treatment and it remained pronounced until the end of the trial. The decrease proved significant when compared with the placebo group. **Stevioside had beneficial effect on the blood pressure and it may help to treat hypertension**. On the other hand, studies on another steviol glycoside, i.e. rebaudioside A, showed no hypotensive effect on humans (Maki, Curry, Carakostas et al., 2008; Maki et al., 2009).

Cardiac hypertrophy, i.e. the thickening of the heart muscle, leads to many cardiovascular diseases associated with hypertension or myocardial infarction. Fan, Lv, Luo, and Tan (2017) induced examined effects of isosteviol (10 μM) on ventricular myocytes isolated from Sprague-Dawley rats' hearts. Hypertrophy in cardiomyocytes was induced by isoproterenol treatment. Isosteviol alleviated the hypertrophy of the rats' cardiomyocytes. The agent regulated L-type Ca-channels and transient outward K-channels and thus prevented longer duration of the action potential in overgrown cardiomyocytes. Isosteviol treatment re-enlarged the cardiac transient outward K-currents, whose substantial decrease was prominent in hypertrophied cardiomyocytes. It also regulated the level of several cardiac hypertrophy markers (atrial natriuretic peptide and brain (B-type) natriuretic peptide). The study suggests that ***Stevia* derivative, isosteviol, may prevent cardiovascular diseases**.

3.5. Anticancer properties

The anticancer properties of steviol glycosides have been described in some preliminary studies. For example, Takasaki et al. (2009) observed that the activation of the Epstein-Barr virus antigen was inhibited by stevioside, steviol and isosteviol. Paul, Sengupta, Bandyopadhyay, and Bhattacharyya (2012) reported that stevioside affected a mitochondria-mediated apoptotic pathway and induced the apoptosis of human breast cancer (MCF-7 cell line) through intracellular ROS generation. Ren, Yin, Yu, and Xiao (2017) observed that stevioside reduced the growth of human colon cancer (HT29 cell line). The growth of human breast cancer cells (MCF-7 Cell Line) was inhibited by steviol (Gupta et al., 2017). The agent affected the cell cycle progression by arresting the G2/M phase. The anticancer effect of steviol was also confirmed in a recent study by Chen et al. (2018). The researchers found that the compound inhibited the growth of human gastrointestinal cancer cells. In this experiment the influence of steviol on the mitochondria-mediated apoptotic pathway was manifested by an increase in the Bax/Bcl-2 ratio (apoptosis marker) and the activation of both the cyclin-dependent kinase inhibitor 1 (p21) and cellular tumour antigen p53 (p53). It is supposed that steviol glycosides exhibit chemopreventive activity and may become an important factor in cancer treatment. Moreover, Deshmukh and Kedari (2014) indicated that methanolic and ethanolic extracts of *Stevia rebaudiana* leaves exhibited anticancer activity in Ca Ski and Caco cell lines. These preparations (25–1000 μg/ml) inhibited the growth of cancer cells in a dose-dependent manner. In conclusion, studies suggest that **steviol glycosides have significant anticancer properties**.

3.6. Antidiabetic properties

In recent years there have been several studies on various experimental models (*in vitro* and *in vivo*), which investigated the antidiabetic potential of *Stevia* preparations. The results revealed the significant antidiabetic potential of *Stevia* and its glycosides and gave further insight into the mechanisms underlying these effects, though they have not been fully understood yet. There have been only a few trials on humans, the most essential of which are presented below.

3.6.1. In vitro models

The *in vitro* models can reveal the mechanisms of action of the tested compounds, but should be carefully interpreted when transferred into living systems. The studies performed on different cell models indicated that steviol glycosides may exhibit appreciable insulin-mimetic activity and antidiabetic properties at some conditions and doses (Rizzo et al., 2013; Mohd-Radzman, Ismail, Jaapar, Adam, & Adam, 2013; Bhasker, Madhav, & Chinnamma, 2015; Prata et al., 2017; Abudula et al., 2008; van der Wielen et al., 2016; Philippaert et al., 2017). The summary of some of those studies are presented below.

Rizzo et al. (2013) showed that **stevioside, rebaudioside A and other steviol glycosides enhanced the glucose uptake as efficiently as insulin**, increased the concentration of glucose transporters GLUT1 and GLUT4 in cells using SH-SY5Y and HL-60 cell lines. A synergistic effect was noted when both steviol glycosides and insulin were applied. Both insulin and the steviol glycoside preparations caused an increase in the phosphorylated forms of phosphoinositide 3-kinases (PI3K) and protein kinases B (Akt).

Mohd-Radzman et al. (2013) reported that stevioside (25–150 μM) **influenced on insulin sensitivity and elevated the glucose uptake** to the degree comparable to rosiglitazone (60–90 μM) in 3T3-L1 cells.

Bhasker et al. (2015) showed that **steviol glycosides intensified activation of the GLUT4 transcript in** 3T3-L1 adipocytes and L6 myotubes (diabetic model cell line).

Abudula et al. (2008) investigated the mechanisms of action of rebaudioside A and stevioside on the glucose metabolism pathway in the pancreatic islets and MIN6 cells isolated from mice. **Rebaudioside A exhibited appreciable insulinotropic effect, but its efficacy was highly glucose-dependent**.

Recent reports have revealed that the transient receptor potential cation channel subfamily melastatin member 5 **(TRPM5) is associated with carbohydrate metabolism**. Philippaert et al. (2017), using human cell line HEK293T and mouse pancreatic islets of male and female C57Bl6/J (WT) and B6.129P2-Trpm5tm1Dgen/J mice, reported that **steviol and stevioside increased the glucose-induced calcium activity in the pancreatic islets and these effects were associated with TRPM5 functions**.

3.6.2. Animal studies

A number of animal studies have been conducted to evaluate the antidiabetic or hypoglycaemic properties of various steviol glycosides, giving contradictory results, depending on the type of compound, its doses and animal model used (Ahmad & Ahmad, 2018; Aranda-González, Moguel-Ordóñez, Chel-Guerrero, Segura-Campos, & Betancur-Ancona, 2016; Dyrskog, Jeppesen, Chen, Christensen, & Hermansen, 2005; Jeppesen et al., 2003; Philippaert et al., 2017; Saravanan & Ramachandran, 2013).

Dyrskog et al. (2005) conducted a trial on diabetic Goto-Kakizaki rats, receiving rebaudioside A (at a dose of 25 mg/kg bw.) in drinking water for 8 weeks. The results **did not show the beneficial effects of the compound on the glucose and insulin levels** on the diabetic animals.

Aranda-González et al. (2016) evaluated the antidiabetic potential of minor steviol glycosides, i.e. dulcoside A, rebaudioside B, C, D and steviolbioside on Wistar rats, treated with the compounds (20 mg/kg bw.) and glibenclamide (5 mg/kg bw.) or metformin (180 mg/kg bw.)

administered with food and intraperitoneally. The tested substances did not have a statistically significant hypoglycaemic effect on rats.

On the other hand, Saravanan and Ramachandran (2013) examined the effects of rebaudioside A on the blood glucose and insulin levels, lipid peroxidation, antioxidative activity and lipid profile during a 45-day experiment on healthy and diabetic Wistar rats. It was found that the treatment with **rebaudioside A improved the blood glucose and insulin levels in diabetic rats. Also, lipid profile, lipid peroxidation biomarkers in the blood plasma and the levels of enzymatic and non-enzymatic antioxidants were regulated.** Moreover, these parameters were comparable to the parameters of healthy rats. This experiment confirmed the beneficial effects of treatment with rebaudioside A on glucose management and lipid metabolism in diabetes.

The preventive effect of stevioside on the development of diabetes was recently examined by Philippaert et al. (2017) using an *in vivo* trial on C57Bl6/J and B6.129P2-Trpm5tm1Dgen/J mice. It was shown that **stevioside significantly decreased hyperglycaemia and the risk of developing glucose intolerance. The effect was highly TRPM5-dependent.** The researchers concluded that the aglycone of steviol glycosides, steviol, may be responsible for the antidiabetic properties of *Stevia*.

3.6.3. Human trials

There have been few studies on the anti-hyperglycaemic potential of *Stevia* and its glycosides conducted on humans. The results are inconsistent or controversial.

For example, Gregersen, Jeppesen, Holst, and Hermansen (2004) conducted a crossover study in which they examined the anti-hyperglycaemic activity of stevioside administered once orally (1 g) in test meals to 12 overweight type 2 diabetic subjects (8 men and 4 women aged approximately 66 years). The results of blood samples drawn before and after the administration showed that **stevioside reduced the postprandial blood glucose and glucagon levels**. Stevioside increased the insulinogenic index ($AUC_{i,insulin}/AUC_{i,glucose}$). The intervention did not alter the urinary excretion of glucose, sodium and potassium. The comparison of both occasions did not reveal statistically significant differences in the blood pressure, insulin, GLP-1, gastric inhibitory polypeptide (GIP), triglycerides and free fatty acid levels. The researchers concluded that stevioside may help to treat type 2 diabetes mellitus.

Ritu and Nandini (2016) conducted a 60-day study on 20 type 2 diabetes mellitus patients to test whether *Stevia* leaf powder administered orally (1 g per day) exhibited hypoglycaemic and hypolipidaemic effects. The results showed that **fasting and postprandial blood glucose levels significantly decreased at the end of the trial in the group treated with the *Stevia* preparation**. Moreover, the ***Stevia* leaf powder lowered the levels of VLDL cholesterol and triglycerides**. The experiment showed the potential of *Stevia* preparations to regulate the biochemical parameters associated with type 2 diabetes mellitus.

On the other hand, Maki, Curry, Reeves et al. (2008) conducted a 16-week trial on 60 overweight patients with type 2 diabetes, but the results of the consumption of **rebaudioside A (1 g per day) did not show any anti-hyperglycaemic effects**. There were no statistically significant differences between the groups in glycaemia, insulin level, C-peptide concentration, body weight, blood pressure and lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, non-HDL cholesterol and total triglycerides). This study showed that rebaudioside A did not have any antidiabetic effect.

Tey, Salleh, Henry, and Forde (2017) conducted a randomised crossover study to examine whether several different low-calorie sweeteners [aspartame (0.44 g per day), monk fruit (50% of mogroside V, 0.63 g per day), *Stevia* sweetener (rebaudioside A, 0.33 g per day) and sucrose (65 g per day)], administered in a beverage instead of sucrose, would affect the energy intake, insulin response and blood glucose levels of healthy men. There were no statistically significant differences in energy consumption between the groups of participants consuming low-calorie products, as they compensated the energy intake with later meals. As expected, the blood glucose and insulin levels increased in response to the high consumption of sucrose, whereas the low-calorie sweeteners did not cause this effect. All the low-calorie beverages had similar and minimal effect on the blood glucose and insulin levels during the day (while eating other meals). However, the most noticeable increase in the insulin level was observed in the *Stevia* beverage group. Also, it resulted in the lowest energy compensation during the day. The researchers concluded that **rebaudioside A may help to treat diabetes not only by improving blood parameters but also by slightly reducing the desire to eat and lowering sugar consumption**. Additionally, the study showed that the glycoside required high plasma glucose concentrations to fully exhibit its antidiabetic effects.

To sum up, unlike the experimental models, the trials conducted on humans to evaluate the anti-hyperglycaemic potential of *Stevia* and its glucosides were insufficient to draw definite conclusions. Further research is necessary.

4. Conclusions

The functional properties of *Stevia* and its glycosides showed their high potential for use in the food industry, mainly as high intensive sweeteners. Moreover, numerous studies on *Stevia* preparations and steviol glycosides conducted on various *in vitro* and *in vivo* models provide increasing evidence that these substances could also be used in a dietotherapy to support certain body functions, particularly to control glucose metabolism in diabetes. However, the mechanisms underlying the antidiabetic action are not fully understood, the dose-response relations for the application to humans have not been fully investigated and require further research.

Ethics statement

This statement refers to Review article. The authors' research did not include any human subjects and animal experiments.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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