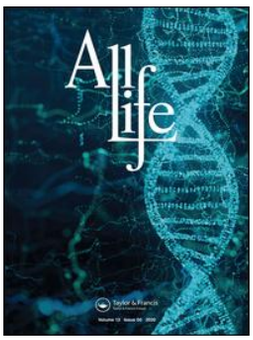

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# 'Sweeter' than its name: anti-inflammatory activities of *Stevia rebaudiana*

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**ABSTRACT**

Inflammation plays a major role in etiology of multiple diseases and it has become an imperative therapeutic target in pharmacological interventions. Over the years, natural products originating from plants have made great contributions in the drug discovery process. Belonging to the *Asteraceae* family, *Stevia rebaudiana* (*S. rebaudiana*) is exploited at a large scale for its purpose as a natural sweetener. Even so, researchers have begun to notice other bioactive potential use of stevia such as anti-inflammatory and anti-cancer activities, which are conferred by compounds present in the leaves including stevioside, rebaudioside and isosteviol. In this review, we provide a brief overview of *S. rebaudiana* plant and its bioactive compounds and highlight their anti-inflammatory potential for therapeutic applications.

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Stevia; natural sweetener; anti-inflammatory; stevioside; isosteviol

## Introduction

Inflammation is a natural response that protects host organisms against external injuries and pathogens (Li et al. 2019). Upon activation, the innate immune system of body defense recruits immune cells to target the inflammation site by the production of pro-inflammatory mediators (Al-Kharashi 2018). These mediators like interleukins (ILs) recruit immune cells to assist in the fight against pathogens, foreign bodies or even cancer cells. However, when the inflammatory process gets out of control, the excess production of pro-inflammatory mediators can induce various acute and chronic human diseases (Medzhitov 2010; Kunnumakkara et al. 2018). Nowadays, the commonly prescribed therapeutic drugs for inflammatory diseases include corticosteroids, immunosuppressive agents and non-steroidal anti-inflammatory drugs. Having said that, the usage of these drugs is frequently associated with some unwanted side effects which significantly limited their usage, including the development of drug tolerance, addiction and gastrointestinal toxicity (Xin et al. 2017). Thus, many researchers have turned to natural resources to hunt for anti-inflammatory agents with higher potency and ideally without toxicity.

Native to South America (Paraguay and Brazil), *Stevia rebaudiana* (*S. rebaudiana*) is a perennial shrub of the *Asteraceae* family (Hossain et al. 2017). The practice of using *S. rebaudiana* leaves as natural sweetener begins among the Paraguayan Indians population; only a few leaves are required to increase the sweetness of drinks like herbal teas (Lewis 1992). Ever since, its usage as a natural sweetener has become popular in various regions of the world, particularly within the Asia region (Figure 1; Ohtani and Yamasaki 2004). Besides being used as natural food additives,

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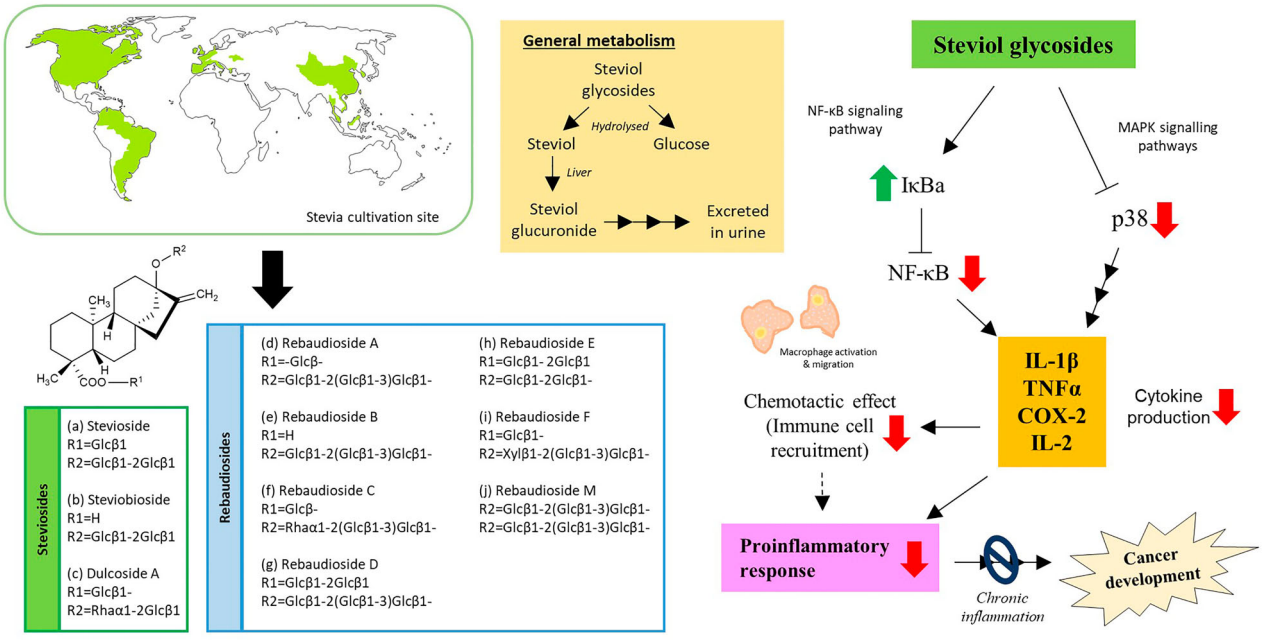


**Figure 1.** General information about native South American plant, *S. rebaudiana*, and its anti-inflammatory activity (adapted from Ferrazzano et al. 2015). The colored regions on the map show cultivation sites of *S. rebaudiana*.

steviol glycosides in *S. rebaudiana* have shown to have high pharmaceutical potential. In fact, evidence is piling up on the potential use of stevia for biological activities, other than its use as a natural sweetener such as anti-cancer, antitumor, immunomodulatory and anti-inflammation without causing any detrimental side effects (Kinghorn 2002; Yasukawa et al. 2002; Gregersen et al. 2004; Chatsudthipong and Muanprasat 2009; Boonkaewwan and Burodom 2013; Wang et al. 2014a; Latha et al. 2017; López et al. 2017; Ruiz-Ruiz et al. 2017; Liu et al. 2018; Casas-Grajales et al. 2019a). Thus, the current review aims to provide an overview on the anti-inflammatory activity of *S. rebaudiana* and highlight its potential as a therapeutic agent against various inflammation-related diseases.

## Botanical description and distribution

Even though the genus *Stevia* contains 230 species, only *S. rebaudiana* gives the sweet essence (Ruiz-Ruiz et al. 2017). Known as 'the sweet herb of Paraguay', *S. rebaudiana* was firstly coinedd by an Italian botanist, Moises S. Bertoni (Soejarto et al. 1983). The plant of *S. rebaudiana* is described as a perennial low shrub with extensive roots, brittle stems and small, elliptical leaves (Table 1) (Ferrazzano et al. 2015; Gutiérrez et al. 2016). Given its economic value as natural sweetener, the cultivation of this plant has exceeded its native habitat (i.e. Paraguay) and it is now widely grown in numerous countries, particularly in India, Japan, Taiwan, Korea, Thailand and Indonesia (Kinghorn 2002; Chatsudthipong and Muanprasat 2009). In fact, the induction load of stevia in India has reached more than 2000 kg/hectare in 2005 with the total annual output is close to 600 tonnes. In terms of growth and

**Table 1.** Botanical classification and morphological features of *Stevia rebaudiana* (modified from Gutiérrez et al. (2016)).

| | | Description |
|---|---|---|
| Botanical classification | Kingdom | Plantae |
| | Subkingdom | Tracheobionta |
| | Superdivision | Sprematophyta |
| | Division | Magnoliophyta |
| | Class | Magnoliopside |
| | Subclass | Asteridae |
| | Group | Monochlamydae |
| | Order | Asterales |
| | Family | Asteraceae (formerly Compositae) |
| | Subfamily | Asteroideae |
| | Tribe | Eupatorieae |
| | Genus | *Stevia* |
| | Species | *rebaudiana* |
| Morphological features | Leaves | Petiole: present, absent; Shape: linear, oblong, elliptical, ovate, rhombic; Margin: entire, subentire, crenate, serrate, dentate; Glands: glandular trichomes |
| | Flowers | Peduncles: shortly pedunculate, sessile; Shape: funnelform; Involucre: cylindrical; Number: 5–6; Color: white, pink, purple |

flowering period, it varies depending on its cultivation site, mainly affected by photoperiod, temperature and water availability of the soil (Ramesh et al. 2006). For example, when cultivated under long-day conditions, *S. rebaudiana* plant produce bigger leaves with a higher content of glycosides (Singh and Rao 2005). These information about growth preference of *S. rebaudiana* is useful to optimize cultivation condition to ensure healthy growth of the plant, and subsequently increase its bioactive compound(s) yield.

## Chemical constituent and extraction of the bioactive compounds from *S. rebaudiana* leaves

*S. rebaudiana* is rich in carbohydrates, protein, crude fiber, minerals and glycosides (Supplementary Table 1; Ijaz et al. 2015). As a matter of fact, the economic value of *S. rebaudiana* heavily relies on the steviol glycosides content in its leaves (Table 2; Figure 1) (Wölwer-Rieck 2012; Aranda-González et al. 2015; Wald and Morlock 2017; Debnath et al. 2018; Gardana and Simonetti 2018; Gomes et al. 2018). The chemical structure of steviol glycoside present as an aglycone (steviol) and three molecules of glucose (Chatsudthipong and Muanprasat 2009). To date, at least 12 steviol glycosides have been identified from stevia leaves, which one of the best-known being stevioside (Rojas et al. 2018). Other steviol glycosides are rebaudioside (A–F), steviolbioside and dulcoside A (Shibata et al. 1995; Rojas et al. 2018). As cultivation conditions may affect the content of glycosides in stevia leaves, the yield of steviosides and rebaudioside A can from 16.3–95 mg/g and 6.86–44.1 mg/g of dry leaves weight, respectively (Wood et al. 1955). Nonetheless, it is worthy to take note that these compounds are at least 50 times sweeter than sucrose, fitting its role as a natural sweetener (Prakash et al. 2008; Ozdemir et al. 2015). At the same time, many research groups have indicated bioactive potentials of these compounds (Geuns 2003; Yadav and Guleria 2012). Therefore, researchers around the world have put in a reasonable amount of effort to develop multiple preservation, extraction and purification strategies to obtain bioactive compounds from the plant (Li et al. 2012; Gallo et al. 2017).

Drying is the first processing stage for stevia leaves, where solar drying is the common practice for preservation purpose. However, solar drying is lacking systemic process control that ultimately affect dried product quality (Lemus-Mondaca et al. 2016). In

**Table 2.** Steviol glycosides present in *S. rebaudiana* leaves (LOD, limit of detection).

| Steviol glycosides | Chemical formula | Molar mass (g/mol) | Amount of steviol glycosides (mg/g DW) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Aranda-González et al. (2015) | Periche et al. (2015) | Lemus-Mondaca et al. (2016) | Wald and Morlock (2017) | Debnath et al. (2018) | Kovačević et al. (2018) | Formigoni et al. (2018) | Gardana and Simonetti (2018) | | Gomes et al. (2018) | Elhassaneen (2019) |
| | | | Mexico | Germany | Chile | Germany | India[a] | Croatia | Brazil | Italy[b] | | Brazil | Egypt |
| Stevioside | $C_{38}H_{60}O_{18}$ | 804.87 | – | 46.48 | 34.4 ± 1.4 | 95 ± 4.0 | 16.3 ± 1.2 | 74.9 ± 1.2–90.9 ± 1.3 | 40.95 ± 1.72 | 0.2 ± 0.01–481.0 ± 17.0 | | 57.09 | 88.7 ± 12.8 |
| Steviol | | | – | – | – | – | – | – | – | – | | – | – |
| Steviolbioside | $C_{32}H_{50}O_{13}$ | 642.73 | 3.5 ± 0.2–5.8 ± 0.1 | – | 20.1 ± 0.8 | 2.0 ± 0.0 | – | – | – | 0.1 ± 0.03–21.0 ± 0.7 | | – | – |
| Rebaudioside A | $C_{44}H_{70}O_{23}$ | 967.01 | – | 17.03 | 11.6 ± 0.5 | 23.0 ± 0.0 | 6.86 ± 0.61 | 21.2 ± 0.4–32.2 ± 1.6 | 44.1 ± 1.85 | 233.0 ± 9.0–994.0 ± 32.0 | | 25.86 | 26.5 ± 2.4 |
| Rebaudioside B[c] | $C_{38}H_{60}O_{18}$ | 804.87 | 2.7 ± 0.1–6.5 ± 0.2 | – | 1.1 ± 0.0 | < LOD | – | – | | 0.1 ± 0.0–256.0 ± 8.0 | | – | – |
| Rebaudioside C | $C_{44}H_{70}O_{22}$ | 951.01 | 20.5 ± 0.1–22.4 ± 0.4 | 6.6 | 1.6 ± 0.0 | < LOD | – | – | 19.91 ± 0.80 | 0.1 ± 0.0–154.0 ± 5.0 | | – | 4.3 ± 1.1 |
| Rebaudioside D | $C_{50}H_{80}O_{28}$ | 1129.15 | – | – | – | < LOD | – | – | – | 0.1 ± 0.0–21.0 ± 0.70 | | – | – |
| Dulcoside A | $C_{38}H_{60}O_{17}$ | 788.87 | 6.1 ± 0.4–7.4 ± 0.3 | 2.03 | 2.1 ± 0.1 | 5.0 ± 1.0 | – | – | – | 0.3 ± 0.01–24.0 ± 0.8 | | – | – |
| Rubusoside | $C_{32}H_{50}O_{13}$ | 642.73 | – | – | 5.3 ± 0.2 | – | – | – | – | 0.3 ± 0.01–23.0 ± 0.6 | | – | – |

[a]Seeds of *S. rebaudiana* were supplied by Mr Andrew Rank (Central Queensland University, Rockhampton, Australia).
[b]The dried Stevia extracts were from European, North American and Chinese suppliers.
[c]It is also known as dulcoside B.

order to extend stevia leaves shelf life and preserving their stability and quality, it is crucial to deliver a quality product with a proper application of drying method and temperature. Numerous drying methods have been developed to obtain the dried stevia leaves with desirable active compounds, namely sun drying, oven drying, microwave drying, shade drying and freeze-drying method. Conventional sun drying method that exposes fresh leaves under direct sun light for 5 days produces sun-dried stevia leaves with the highest moisture, ash, protein and fat content (Gasmalla et al. 2014). Under a range of relatively stable temperature (38.5–58.5 °C) and air humidity (11.5–53.5%), sun-dried stevia leaves extract shows strong anti-inflammatory response against phorbol 12-myristate 13-acetate (TPA)-induced inflammation in ear edema model (Lemus-Mondaca et al. 2018). The microwave drying method is fast and effective against inflammation. It retains the highest amount of carbohydrate, reducing sugar and higher pH values in dried leaves when compared with sun-dried and oven-dried methods (Gasmalla et al. 2014). This method dries leaves between 6 and 8 min at 700–800 W, and it yields a potent anti-inflammatory extract in both arachidonic acid (AA)-induced and TPA-induced ear edema inflammation models (Gasmalla et al. 2014; Lemus-Mondaca et al. 2018). On the other hand, shade drying, hot air drying and freeze drying have no effect on rebaudioside A and C concentration (Periche et al. 2015). These three drying processes have been shown to reduce stevioside content while increase dulcoside A content; nonetheless both compounds achieved the highest value in shade drying method (Periche et al. 2015). A 3-min of hot air-drying process at 180°C also gives the highest content of total phenol, flavonoids and antioxidants in dried stevia leaves (Periche et al. 2015; Lemus-Mondaca et al. 2016). Lemus-Mondaca et al. (2016)'s work on the effect of hot air drying at 30, 40, 50, 60, 70 and 80°C under a constant air velocity of $2.0 \pm 0.2$ m/s on fresh stevia leaves showed that 40°C is the optimal drying temperature to yield high steviol glycosides content (stevioside, steviolbioside, rebaudioside A, B and C, rubusoside and dulcoside) with best antioxidant activity compared to other temperatures. They also showed that hot air-drying temperatures at 60°C and above has a negative impact on the total phenolic and flavonoid. Later, they tested seven drying methods (freeze-drying, vacuum drying, infrared drying, shade drying, sun drying, microwave drying and hot air drying) on fresh stevia leaves and found that freeze-drying method produced the highest antioxidant capacity, higher total polyphenol, total flavonoids content and phenolic compounds (chlorogenic acid, caffeic acid and trans-ferulic acid) when compared with other drying methods and fresh stevia leaves extract (Lemus-Mondaca et al. 2018). Other than ther infrared drying method, the rest of the drying methods reduced inflammation in ear edema models; vacuum drying and shade drying methods showed the strong anti-inflammatory effect on AA-induced and TPA-induced inflammation, respectively (Lemus-Mondaca et al. 2018). Taken together, drying method and temperature on fresh stevia leaves are important to generate the leaves extract for desirable bioactivity or compound isolation.

In terms of extraction, one of the conventional methods involves the use of organic solvents such as chloroform–methane, glycerol and propylene glycol (Kinghorn and Soejarto 1985; Pasquel et al. 2000). Taking into considerations that some organic solvents may not be environmentally friendly and/or poses some health hazards to operators, researchers have suggested the use of water to be used instead as stevioside is water-soluble too (Phillips 1987; Bovanová et al. 1998; Vaněk et al. 2001; Pól et al. 2007). As extraction methods evolved, more sophisticated techniques have been incorporated into the extraction process of stevioside, including pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) (Tan et al. 1988; Kienle 1990; Kovylyaeva et al. 2007; Liu et al. 2010). By the same token, these techniques offer greater advantages over conventional solvent extraction systems. For instance, the typical solvent volume required for MAE is about 10 ml/g of plant sample, which is much lesser than that required in conventional extraction methods (Soxhlet extraction: 30 ml/g of plant sample) (Javad et al. 2014). Jaitak et al. (2009) compared three different extraction methods – conventional solvent extraction, MAE and UAE. Based on their results, MAE yielded highest stevioside (8.64%) and rebaudioside A (2.34%), followed by conventional extraction (stevioside 6.54%; rebaudioside A 1.20%) and UAE (stevioside 4.20%; rebaudioside A 1.98%). A strong evidence presented by the same team emphasized the advantage of MAE and UAE over conventional solvent extraction is the time required for the

extraction. The whole extraction process with MAE is much quicker, dropping extraction time from 16 h (using Soxhlet extraction) to just a few minutes – ∼30 min for UAE and only 1 min for MAE (Jaitak et al. 2009; Ferrazzano et al. 2015). Similarly, a research team from Singapore compared the extraction efficiency of MAE to another modern green approach known as PHWE, which use superheated or sub-critical water to extract steviol glycosides present in stevia leaves (Teo et al. 2009). Even though the team had successfully extracted steviosides using PHWE, MAE appeared to be a better choice with higher extraction efficiency as PHWE requires temperature optimization to ensure good yield (Teo et al. 2009; Kovačević et al. 2018). Similar results were reported by Periche et al. (2015) which compared three different methods – conventional (heating) method, MAE and UAE. Compared to conventional method, MAE and UAE extracted higher yield of different steviol glycosides – (a) Dulcoside A: 2.03 and 2 mg/g, (b) Rebaudioside A: 17.03 and 14.12 mg/g, and (c) Rebaudioside C: 6.6 and 6.25 mg/g (d) Stevioside: 46.48 and 39.06 mg/g, respectively.

Aside from these techniques, SFE is another extraction method deployed to obtain steviol glycosides from stevia plant, yet studies have expressed 'mixed feelings' over the extraction efficacy of this technique (Pasquel et al. 2000; Choi et al. 2002; Yoda et al. 2003; Erkucuk et al. 2009; Ameer et al. 2017). For instance, the total yield of glycosides obtained by Erkucuk and team using SFE (45.13–54.26 mg/g) was similar to those obtained using conventional water extraction (64.49 mg/g) and slightly higher than ethanol extraction (48.60 mg/g), thus implying the cost-effectiveness of SFE for industrial-scale application. On the other side, Ameer et al. (2017) demonstrated that the optimization of SFE via response surface methodology and artificial neural network modeling could generate higher targeted extraction (i.e. steviol glycosides at) than conventional extraction, offering a faster, lower energy, and eco-friendly extraction method with lesser $CO_2$ emissions and reduced solvent use.

Another important factor for practical and industrial applications is the purity of acquired bioactive compound, particularly eliminating the signature bitter-off taste of stevia. For this reason, Zhang et al. (2000) developed a membrane chromatography technique to obtain high purity steviol glycoside while removing most of the bitter-tasting components. Lately, a team from Italy has described a newer solid–liquid extraction known as rapid solid–liquid dynamic extraction (RSLDE) (Gallo et al. 2017; Gallo et al. 2018). Compared to the Soxhlet method, this technique is a much 'greener' option, operating at low (room) temperatures while maintaining extraction reproducibility (Gallo et al. 2017; Naviglio et al. 2019). For the extraction of stevia, Gallo et al. (2017, 2018) discovered that 'complete' extractions of rebaudioside A and stevioside can be achieved in 40 min. Compared to traditional heating method or maceration, the leaves were more intact and remained in a filter bag, bypassing the need of additional filtering steps to remove solid materials and more suitable for further use in agronomy and animal nutrition (Gallo et al. 2017, 2018). In truth, Phillips (1987) has suggested a pre-treatment with solvents like chloroform or hexane prior to actual extraction processes to remove other essential oils, lipids, chlorophyll and other non-polar substances during the late 1980s. Besides reducing impurities, Formigoni et al. (2018) have shown that the pre-treatment (with ethanol) increased the yield of steviol glycosides from stevia leaves, reduced its bitter aftertaste while achieving sensory profile similar to sucralose. Along with this, some researchers have also pointed out that using solvent alone may not be able to penetrate into the sample core to ensure complete release target compounds, which in turn result in lower extraction efficiency (Teo et al. 2009). Pulsed electric fields (PEF) technology and high-voltage electrical discharges (HVED) are examples of highly innovative extraction methods that involve electrical treatments of short time (from several nanoseconds to several milliseconds) to electrically 'pierced' biological membrane, enabling higher extraction and recovery of compound of interest (Barba et al. 2015a, 2015b; Carbonell-Capella et al. 2017; Li et al. 2019). Both PEF and HVED improved the extraction of rebaudioside A and stevioside compared to traditional grinding, shortening 'buffer time' before subsequent purification and concentration steps (Barba et al. 2017). Coupled with analytical methods like ultraviolet and visible spectrophotometer (UV–Vis) and Fourier transform infrared spectroscopy (FTIR), the extraction and identification technologies of the chemical constituent present in *S. rebaudiana* have progressed much since the discovery of the plant, enabling higher efficiency and faster access for future drug development work.

## Metabolism of stevioside in the body system

In general, *S. rebaudiana* plants have long been used by the Paraguayan populations with no toxicity reported previously (Curry and Roberts 2008; Momtazi-Borojeni et al. 2017). Stevioside was found to bear a very low acute oral toxicity ($LD_{50}$ between 8.2 and 17 g/kg) in the mouse, rat and hamster (Medon et al. 1982; Toskulkac et al. 1997; Sharma et al. 2009; Brahmachari et al. 2011). Furthermore, no carcinogenicity was observed in the standard AMES test and rodent models (Hagiwara et al. 1984; Suttajit et al. 1993; Toyoda et al. 1997; Eriko et al. 2003). The European Food Safety Authority (EFSA) has published a scientific report in 2015, highlighting that stevioside is neither carcinogenic nor genotoxic with no risks associated with reproductive or developmental toxicity (EFSA ANS Panel 2015). When ingested, steviol glycoside is not digested by human but rather hydrolyzed by the gut microbes into steviol, by cutting off their glucose units (Koyama et al. 2003; Geuns et al. 2007; JECFA 2008; Wheeler et al. 2008; Ashwell 2015). Consequently, steviol is absorbed via the portal vein before undergoing glucuronidation process to yield the final metabolite steviol glucuronide, which is then excreted in the urine (Wingard et al. 1980; Koyama et al. 2003; Roberts and Renwick 2008). Looking at pharmacokinetics data, Wheeler et al. (2008) have reported that steviol glucuronide was detected in the plasma of all human subjects after administration of rebaudioside A (5 mg/kg) or stevioside (4.2 mg/kg), with the median time taken to reach the maximum concentration ($T_{max}$) values of 12 and 8 h post-dose, respectively. In the same study, no safety concerns or adverse events were reported. With the growing evidence supporting the safety of stevia use, World Health Organization (WHO) approved stevia as a food additive in 2004 with a recommended acceptable daily intake (ADI) to be up to 4 mg/kg body weight (JECFA 2008). Likewise, the use of stevioside as food additive is also approved under the General Standard for Food Additives (GSFA), in countries like the USA and Canada (Martyn et al. 2018; Perrier et al. 2018). Nevertheless, a study by Robert et al. in 2016 claimed that the current ADI is assumed as particularly conservative; using rat and human as study subjects, the group proposed that the higher ADI should be implemented, falling between 6 and 16 mg/kg body weight per day. Altogether, these studies supported the safe use of stevia and its metabolites, which subsequently promote its potential to be exploited for pharmaceutical use.

## Use of stevia as 'top-pick' for sugar substitute in metabolic diseases

Coming with a zero calories content, stevia represents a good and healthy substitute for sugars, particularly for diabetic patients. Diabetes mellitus (DM) is a multifaceted metabolic disease characterized by glucose dysregulation which results in hyperglycemia (Care 2006). Type 1 (T1D) and type 2 (T2D) diabetes are categorized by their pathogenesis, where T1D involves immune-modulated pancreatic beta cells destruction while T2D primarily demonstrates insulin resistance and deficiency (Tsalamandris et al. 2019). Piling evidence have associated inflammation with the progression of DM via detection of circulatory inflammatory markers such as IL-6 in epidemiological studies and animal models (Table 3) (Duncan et al. 2003; Marques-Vidal et al. 2012; Tsalamandris et al. 2019). Insulin resistance and deficiency in pancreatic $\beta$ cells insulin secretion are considered as pro-inflammatory states as they have close links with inflammatory responses (Hotamisligil et al. 1993; Lohner et al. 2017; Zhong et al. 2017). Stevia and its extracts are known to possess insulinotropic, glucagonostatic, antihyperglycemic and blood-pressure-suppressing effects in multiple DM models (Suanarunsawata et al. 2004; Holvoet et al. 2015; Latha et al. 2017). Stevioside treatment at 25, 30 and 300 mg/kg on Goto-Kakizaki rats (a non-obese genetic T2D experimental model) have consistently displayed higher first-phase insulin responses and lower glucagon level during insulin-assisted glucose tolerance tests (IAGT) (Jeppesen et al. 2002, 2003, 2006). It significantly reduces hyperglycemia condition, both systolic and diastolic blood pressure, as well as plasma cholesterol level when administered for 5 weeks (Jeppesen et al. 2002, 2003, 2006). Administration of stevia leaves extracts, leaves powder and also the extracted polyphenols improves and alleviates DM symptoms such as water intake and body weight gain while protecting the kidney, pancreas and liver organs in both streptozotocin (STZ)-induced (Suanarunsawata et al. 2004; Chang et al. 2005; Sumon et al. 2008; Ozbayer et al. 2011; Saravanan et al. 2012; Shivanna et al. 2013; Akbarzadeh et al. 2015; Assaei et al. 2016; Perumal et al. 2016; Ahmad and

**Table 3.** Use of stevia leaves and its bioactive compounds in diabetes animal model (N.S., not stated; N.R., not relevant; IVGT, intravenous glucose tolerance; IAGT, intra-arterial glucose tolerance; IPGT, intraperitoneal glucose tolerance; IAGT, intra-arterial glucose tolerance; OGTT, oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GSH, reduced glutathione; SOD, superoxide dismutase; TG, triglycerides).

| No. | Reference | Plant part | Tested substance | Animal model | Inducer(mg/kg) | Findings |
|---|---|---|---|---|---|---|
| [1] | Jeppesen et al. (2002) | N.R. | Stevioside (200 mg/kg) | Goto-Kakizaki and Wistar rats | N.R. | • During IVGT test, stevioside significantly suppressed glucose response and glucagon levels, but concomitantly increased insulin response in GK rats<br>• In normal Wistar rats, stevioside enhanced insulin response without affecting glucagon levels and glucose response<br>• Stevioside exerted antihyperglycaemic, insulinotropic, and glucagonostatic actions in the type 2 diabetic rat model |
| [2] | Jeppesen et al. (2003) | N.R. | Stevioside (25 mg/kg) | Goto-Kakizaki and Wistar rats | N.R. | • During IAGT test, stevioside reduced hyperglycemia condition, by enhancing first-phase insulin response and suppressing glucagon levels<br>• Stevioside significantly suppressed systolic and the diastolic blood pressure |
| [3] | Jeppesen et al. (2006) | N.S. | Stevioside (30 mg/kg) | Goto-Kakizaki rats | N.R. | • During glucose tolerance test, stevioside-treated animals showed much higher first-phase insulin response and lower glucagon level compared to control animals<br>• Stevioside lowered plasma cholesterol level after 5 weeks of treatment |
| [4] | Suanarunsawata et al. (2004) | Leaves | Aqueous extract (250 mg/kg), Stevioside (250 mg/kg) | Wistar rats | Streptozotocin (40) | • Aqueous extract increased insulin level, decreased plasma glucagon level and reduced plasma glucose level in the diabetic group<br>• Insulin-induced glucose uptake study using isolated diaphragm muscle revealed suppression action by stevioside in both normal and diabetic rats<br>• Stevioside present in stevia extract may indirectly contribute to antihyperglycemic action via potentiating insulin release |
| [5] | Sumon et al. (2008) | Leaves | Aqueous extract (150, 200, 250 mg/kg) | Albino rats | Streptozotocin (55) | • Stevia leaves powder significantly reduced blood glucose levels and possessed hypoglycemic effect at all concentrations |
| [6] | Saravanan et al. (2012) | N.R. | Rebaudioside A (50, 100, 200 mg/kg) | Albino Wistar rats | Streptozotocin (40) | • Rebaudioside A decreased blood glucose, increased the level of plasma insulin and attenuated pancreas damage<br>• Reduced activities of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase while increased hexokinase and glucose-6-phosphate dehydrogenase in the liver along with glycogen in a significant manner |

(*continued*).

**Table 3.** Continued.

| No. | Reference | Plant part | Tested substance | Animal model | Inducer(mg/kg) | Findings |
|---|---|---|---|---|---|---|
| [7] | Shivanna et al. (2013) | Leaves | 4% stevia leaves powder, extracted polyhenols and extracted fiber from 4% stevia leaves powder[a] | Wistar rats | Streptozotocin (60) | • Stevia leaves powder and extracted polyphenols reduced food and water consumption, urine output, blood glucose level while increased serum insulin level<br>• Protect against streptozotocin-induced kidney damage and liver damage via decreased glomerular filtration rate and serum level of liver ALT and AST enzymes<br>• Antioxidant properties via increased SOD and catalase enzyme activity while reduced lipid peroxidation<br>• No changes in stevia fiber-fed rats |
| [8] | Akbarzadeh et al. (2015) | N.S. | Aqueous extract (250, 500, 750 mg/kg) | Wistar rats | Streptozotocin (60) | • Stevia extracts (at a dose rate of 250 and 500 mg/kg) reduced fasting blood sugar, TG, omentin, alkaline phosphatase levels, without changing the number of pancreatic $\beta$-cells<br>• Stevia extracts decreased the omentin level, possibly by indirectly activating insulin sensitivity and lowering blood glucose |
| [9] | Assaei et al. (2016) | Leaves | Aqueous extract (400 mg/kg) | Sprague–Dawley rats | Streptozotocin (40) | • Aqueous extracts reduced fasting blood sugar, TG, malondialdehyde, ALT and AST levels<br>• Normalized lipid peroxidation and catalase activity in the pancreas of the diabetes group treated with stevia (i.e. increased catalase activity and decreased MDA levels compared to the diabetic model group)<br>• Aqueous extracts enhanced PPAR$\gamma$ and insulin mRNA levels<br>• Histological analysis showed that groups treated with aqueous extracts have relatively better synthesis and aggregation of insulin in the pancreas |
| [10] | Perumal et al. (2016) | Leaves | Hydro-methanol extract-loaded chitosan nanoparticles (100 mg/kg) | Wistar rats | Streptozotocin (55) | • Stevia extract-loaded chitosan nanoparticles reduced fasting blood glucose level<br>• Improved diabetic biochemical profiles by reduced glyoxylate hemoglobin level, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase and alkaline phosphatases<br>• Normalized liver and kidney lipid peroxidation and antioxidant activity of CAT, GSH and SOD.<br>• Alleviated streptozotocin-induced pancreatic and liver damage via restoration of hyperplatic islets of Langerhans, hepatocyte hypertrophy and lymphocyte infiltration in the liver |

| | | | | | | |
|---|---|---|---|---|---|---|
| [11] | Ahmad and Ahmad (2018) | Leaves | Aqueous extract (200, 300, 400, 500 ppm/kg) | Albino rats | Streptozotocin (40) | • Stevia extract reduced feed and water intakes as well as body weight in diabetic rats<br>• Stevia extract decreased blood glucose (random and fasting) and glycosylated hemoglobin level in diabetic rat, which is consistent with increased level of serum insulin and liver glycogen levels |
| [12] | Ozbayer et al. (2011) | N.S. | Stevia extract (200 mg/kg) | Sprague–Dawley rats | Streptozotocin (60)–nicotinamide (290 | • Stevia extract increased oxide synthase activity in the kidney<br>• Stevia decreased urine glucose level, thereby decreasing urine pH in diabetic rats |
| [13] | Aranda-González et al. (2016) | N.R. | Rebaudioside B, Rebaudioside C, Rebaudioside D, Dulcoside A, Steviolbioside (20 mg/kg) | Wistar rats | Streptozotocin (65)–nicotinamide (120) | • Both acute intraperitoneal and chronic oral administration of rebaudiosides B, C, D, Dulcoside A and Steviolbioside did not exert antihyperglycemic acitivity in IPGT test |
| [14] | Elhassaneen (2019) | Leaves | Mixture powder (1, 2, 3 and 4% w/w) | Albino rats | Streptozotocin (40) | • Stevia leaves powder significantly decreased fasting serum glucose concentration and thiobarbituric reactive substances level in dose-dependent manner |
| [15] | Kujur et al. (2010) | Leaves | Aqueous, methanolic, ether extracts (2000 mg/kg) | Wistar rats | Alloxan (125) | • All stevia extracts significantly decreased the blood glucose level in diabetic rats as tested on the 28th day |
| [16] | Misra et al. (2011) | Leaves | Benzene:acetone extract (200 and 400 mg/kg)[b] | Albino Wistar rats | Alloxan monohydrate (180) | • Stevia extract-treated rats attained nearly normal blood glucose level on the 10th day<br>• Significant ($p < .01$) dose-dependent reduction in body weight of alloxan-induced diabetic rats was observed after stevia extract treatment |
| [17] | Hossain et al. (2011) | Leaves | Petroleum ether, ethyl acetate and chloroform extracts (150 mg/kg)[d] | Long–Evans rats | Alloxan (110) | • All three extracts (petroleum ether, ethyl acetate and chloroform) reduced the severity of hyperglycemia, enhanced antihyperlipidemic activity and improved glucose tolerance activity |
| [18] | Sharma et al. (2012) | N.S. | Commercial stevia extract, HERBOCAL (250 mg/kg) | Wistar rats | Alloxan (150) | • Stevia extract reduced blood glucose level in diabetic rats, while increased levels of antioxidant enzymes (CAT, SOD, GSH) in the liver and inhibited lipid peroxidation |
| [19] | Raskovic et al. (2004) | N.R. | Stevita (200 mg/kg), Clear stevioside liquid (20 mg/kg)[c] | NMRI Haan mice | Alloxan (100) | • Mice pre-treated with Stevita and stevioside did not exhibit significant increase in glycemia after two doses of alloxan<br>• Blood glucose concentration of groups pre-treated with stevia and stevioside was lower than control |

(*continued*).

**Table 3.** Continued.

| No. | Reference | Plant part | Tested substance | Animal model | Inducer(mg/kg) | Findings |
|---|---|---|---|---|---|---|
| [20] | Singh et al. (2013) | Leaves | Methanolic extract (300 mg/kg) | Swiss albino mice | Alloxan monohydrate (150) | • Group treated with methanolic extract for 21 days showed significant reduction in blood glucose |
| [21] | Ilić et al. (2017) | N.R. | Stevioside hydrate (20 mg/kg) | NMRI Haan mice | Alloxan (150) | • Compared to the diabetic group, stevioside solution prevented the increase in blood glucose during OGTT<br>• Stevioside administration conferred certain protective effects against alloxan-induced oxidative damage of the pancreatic $\beta$-cells as shown in histopathological studies |
| [22] | Perumal et al. (2016) | Leaves | Stevioside (0.5, 1.0, 5.0 mg/kg) | Wistar rats | Streptozotocin (60)<br>Fructose-rich feed | • Stevioside improved insulin response<br>• IPGTT test showed that stevioside reduced plasma glucose (in a dose-dependent manner) and body weight |
| [23] | Dyrskog et al. (2005) | N.R. | Steviosides (91%), Stevioside supplement (Pure Stevioside, 4% Rebaudioside A, and 5% other glycolsides) (30 mg/kg) | Type 2 diabetic Zucker diabetic fatty rats | N.R. | • Combination of stevioside supplement and soy protein lowered blood glucose in IAGT test<br>• The combination reduced systolic blood pressure and improved the blood lipid profile after 2 weeks of administration |
| [24] | Nordentoft et al. (2008) | N.R. | Isosteviol (20 mg/kg) | Diabetic KKAy mice | N.R. | • Isosteviol improved glucose homeostasis, increased insulin sensitivity, lowered plasma triglycerides and lowers weight<br>• Regulated the gene expression level of key insulin regulatory genes GLUT2, Ins1, Ins2, Pdx1/Ipf1, Beta2/Neurod1, Pax6 and11-beta-HSD-1 and beta-cell transcription factors Nkx2-2, Nkx6-1, C/EBPalpha and FoxA2 |
| [25] | Elnaga et al. (2016) | N.R. | Pure stevia sweetener (25, 250, 500, 1000 mg/kg) | Overweight Wistar strain rats | N.R. | • Stevia leaves powder and extracted polyphenols reduced food and water consumption, urine output, blood glucose level while increased serum insulin level.<br>• Protect against streptozotocin-induced kidney damage and liver damage via decreased glomerular filtration rate and serum level of liver aminotransferase enzymes<br>• Antioxidant properties via increased SOD and CAT enzyme activity while reduced lipid peroxidation.<br>• No changes in stevia fiber-fed rats |
| [26] | Aghajanyan et al. (2017) | N.S. | Aqueous extract (100 mg/kg) | Rabbits | Immobilization stress-induced hyperglycemia | • Aqueous extract reduced fasting glucose level<br>• Lowered atherogenic index via reduced levels of total cholesterol, TG and LDL-cholesterol while increased HDL-cholesterol level<br>• Increased liver and muscle glycogen level<br>• No differences in body weight and food consumption |

[a] 4.0% stevia leaves powder incorporated diet (4.0 g leaf powder in 96 g dry diet); with equivalent amount of polyphenols extract (through force feeding); with equivalent amount of fiber extracted from 4 g of stevia leaves powder.

[b] Mean stevioside content was determined to be 0.454% dry leaves weight.

[c] This study is a pre-treatment study using commercially available stevia products from Stevita Co, INC, Arlington Texas and Stevita Co, INC, Herbal supplement, Brazil.

[d] These extracts were derived from ethanolic fraction of stevia leaves.

Ahmad 2018; Elhassaneen 2019) and alloxan-induced DM rat/mouse models (Raskovic et al. 2004; Kujur et al. 2010; Hossain et al. 2011; Misra et al. 2011; Sharma et al. 2012; Singh et al. 2013; Ilić et al. 2017), as well as T2D Zucker fatty rats (Dyrskog et al. 2005), KKAy mice (Nordentoft et al. 2008) and immobilization stress-induced hyperglycemic rabbits (Aghajanyan et al. 2017). Besides lowering blood (random and fasting) and urine glucose levels, reducing blood pressure, as well as elevating plasma insulin level and improves biochemical profiles of triglycerides (TG), stevia extracts increases kidney nitric oxide synthase (NOS), liver catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) activities while normalizing elevated pancreatic lipid peroxidation (Ozbayer et al. 2011; Sharma et al. 2012; Shivanna et al. 2013; Akbarzadeh et al. 2015; Assaei et al. 2016; Perumal et al. 2016). These beneficial effects may result from the regulatory role of stevia and its extracts on key insulin regulatory genes (GLUT2, Ins1, Ins2, Pdx1/Ipf1, Beta2/Neurod1, Pax6 and11-beta-HSD-1) and pancreatic beta-cell transcription factors (Nkx2-2, Nkx6-1, C/EBPalpha and FoxA2) observed in isolated islets of the KKAy mice (Nordentoft et al. 2008). On its insulinotropic effect, stevioside modulates insulin release by potentiating the calcium ion-dependent activity of TRPM5 ion channel and augmenting glucose-induced calcium ion oscillations in pancreatic islets (Philippaert et al. 2017). Like stevia and its extracts, Rebaudioside A also shows blood glucose-lowering, glucagonostatic and insulinotropic effects on STZ-induced diabetic rats (Saravanan et al. 2012). On the contrary, both acute intraperitoneal and chronic oral administration of rebaudiosides B, C, D, dulcoside A and steviolbioside do not exert antihyperglycemic acitivity in intraperitoneal glucose tolerance test (IPGTT) examined on STZ-induced diabetic rats (Aranda-González et al. 2016). In the context of the anti-inflammation activity, a higher PPAR$\gamma$ gene expression is reported in aqueous stevia extract-treated STZ-induced diabetic rats (Assaei et al. 2016). PPAR$\gamma$ is a glucose metabolism regulator and an established anti-inflammatory player that negatively regulates macrophages and inhibits pro-inflammatory cytokines production (Jiang et al. 1998; Reddy et al. 2008; Ahmadian et al. 2013). One study on non-obese diabetic mice with Sjogren's syndrome showed that PPAR$\gamma$ treatment significantly ameliorated lymphocytes infiltration and inflammatory responses in salivary glands (Li et al. 2014). Stevia too alleviates STZ-induced lymphocyte infiltration in the liver (Perumal et al. 2016) which may indicate the involvement of PPAR$\gamma$ pathway in its anti-inflammatory effect. Besides PPAR$\gamma$ activation, PI3K/Akt pathway is also a potential anti-inflammatory mechanism for stevia and its extracts as steviol glycosides induces Glut4 translocation to the plasma membrane by activating the PI3K/Akt pathway then further increase glucose uptake into rat fibroblasts (Prata et al. 2017). Along with these findings, researchers have discovered that stevia offers much more benefits than just antihyperglycemic in diabetic patients, particularly in improving wound healing and prevent scar formation (Gans 2003; Das 2013; Babakhanyan et al. 2017; Siraj et al. 2019). Individuals suffering from DM often present delayed healing, or even formation of necrotic tissues. Firstly, bacteriostatic properties of stevioside would prevent wound colonization and infection by harmful pathogens, thereby highlighting its potential in regulating immunoresponse and improving vascularity promote wound closure and healing (Babakhanyan et al. 2017).

Besides that, there are few reports highlighting the protective and preventive properties of stevia against atherosclerosis, which was formerly considered as a bland lipid storage disease accompanied with the apparent inflammatory response (Ross 1999; Libby et al. 2002). As stevia can improve serum lipid profiles such as higher high-density lipoprotein (HDL) level and lower total lipids, total cholesterol, triglycerides and low-density lipoprotein (LDL) levels in overweight rats and immobilization stress-induced hyperglycemic rabbits (Elnaga et al. 2016; Aghajanyan et al. 2017), studies have been carried out to examine its anti-atherogenic effects. Aqueous stevia leaves extract shows its anti-atherogenic effect by inhibiting $CuSO_4$-induced LDL oxidation, which is a part of the pathogenesis of atherosclerosis (Elhassaneen 2019). Stevioside (10 mg/kg) administration also reduces plaque volume in the aortic arch of obese insulin-resistant mice by lowering the macrophage, lipid and oxidized LDL content in the plaque (Geeraert et al. 2010). Its antioxidant effect on both the adipose tissue and the vascular wall induces plaque stabilization while inhibiting atherosclerotic plaque development in the same study. Doing more good than harm, the stevia plant and its steviol glycosides appear to be an

attractive source for anti-inflammatory agent, so as to expand its applications beyond metabolic disorders such as DM and cardiovascular diseases.

## More than just sweetener: anti-inflammatory activity of *S. rebaudiana*

Over the years, inflammation has been recorded as the key player in the development of various human diseases including arthritis, inflammatory bowel disease and atherosclerosis (Shoelson et al. 2006; Audial and Bonnotte 2015). The development of the natural product, like steviol glycosides, for the prevention of pro-inflammation process, may essentially be developed as a safe, efficient and cost-effective pharmaceutical intervention (Roberts and Munro 2009; Auyeung et al. 2016). Several studies have indicated the anti-inflammatory properties of stevia and its components using both *in vitro* and *in vivo* models (Table 4). For instance, numerous groups began their work by studying anti-inflammatory activity of stevia extracts. Few reports showed that hydroalcoholic extract of stevia leaves (500 mg/kg) can prevent inflammation and also reduce oxidative damage in the liver, predominantly via altering the level of pro-inflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$ and IL-6 (Holvoet et al. 2015; Latha et al. 2017). Generally, cytokines are produced as important signaling molecules during physiological and pathological processes by immune cells like macrophages, lymphocytes and various stromal cells. One of the studies showed that stevioside can suppress the secretion of pro-inflammatory cytokines in macrophages after being challenged with lipopolysaccharides in a dose-dependent manner (Fengyang et al. 2012). Similar observations were reported by Meng et al. (2018), whereby the group stated that stevioside at 200 μM suppressed titanium particle-induced inflammatory response in bone marrow-derived macrophages and prevented osteolysis in mice treated with titanium particles (with a dose of 10 or 30 mg/kg). Based on their findings, stevioside conferred anti-inflammatory activity via downregulation of two major pathways, mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-$\kappa$B) signaling pathways. Similar findings were also reported by another team in Thailand, which explained the stevioside suppressed pro-inflammatory cytokines release of immune cells (i.e. TNF-$\alpha$, IL-1$\beta$ and IL-6), and eventually inhibited NF-$\kappa$B pathways and activated of its inhibitor, I$\kappa$B$\alpha$ protein (Boonkaewwan et al. 2006; Boonkaewwan and Burodom 2013). Comparably, stevioside was able to reduce inflammation in a *Staphylococcus aureus*-infected mouse model via actions on MAPK and NF-$\kappa$B pathways (Wang et al. 2014a, 2014b). The research group initially conducted a study on primary mouse mammary epithelial cells (MMECs) infected with *S. aureus* and discovered that stevioside was able to inhibit the secretion of inflammatory cytokines like TNF-$\alpha$, IL-1$\beta$ and IL-6, probably via actions on MAPK and NF-$\kappa$B pathways. Besides effectively assisting in the treatment of *S. aureus*-induced mastitis, stevioside was able to prevent cell death of the *S. aureus*-infected MMECs by downregulating the expression of type 2 Toll-like receptors (TLRs), which is a key player in regulating inflammation and apoptosis (Wang et al. 2014a). Taking this information further, Wang and his team have investigated the anti-inflammatory potential of stevioside in the *S. aureus*-infected mouse mammary gland by administering the bacteria intraperitoneally (Wang et al. 2014b). Through this study, it was unveiled that stevioside reduced inflammatory cell infiltration and preserved the histological structure of the mammary glands (e.g. complete lobules, acinus). These findings were consistent with those from primary culture, stevioside was shown to possess protective and anti-inflammatory effects against S. *aureus*-infected mouse mammary gland, via regulatory actions on TLR2 expression, cytokines and proteins of the NF-$\kappa$B and MAPK pathways. Collectively, these results suggest that stevia and its important constituent, stevioside prevents inflammation to occur by inhibiting the production of cytokines in immune, stromal and/or epithelial cells via downregulation of MAPK and NF-$\kappa$B signaling pathways.

Apart from that, the accumulation of free radicals often causes detrimental effects to the host. Free radicals can modify or damage biological macromolecules such as protein, lipid and DNA. These damages consequently compromise the functioning of crucial processes including DNA repair system, which ultimately leads to mutation or even development of cancer (Pacifici and Davies 1991; Bartsch and Nair 2006; Klaunig 2018). Stevioside has been shown to be able to reduce oxidative stress and inflammation by several mechanism of actions. Ramos-Tovar et al. (2018a) revealed that stevia extract (100 mg/kg) and stevioside (100 mg/kg) can prevent oxidative damage

**Table 4.** Anti-inflammation activity of stevioside and related compounds (N.S., not stated; N.R., not relevant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; $ID_{50}$, half maximum inhibitory dose; LPS, lipopolysaccharides; qRT-PCR, quantitative real-time PCR; MPO, Myeloperoxidase; ELISA, enzyme-linked immunosorbent assay; IHC, Immunohistochemistry; Nrf2, nuclear factor (erythroid-derived 2)-like 2; TAA, thioacetamide).

| No. | References | Plant part | Tested substance | Experimental design | Dosage and results |
|---|---|---|---|---|---|
| [1] | Yasukawa et al. (2002) | N.R. | Stevioside[a], Rebaudioside A[a], Rebaudioside C[a], Dulcoside A[a], Stevioside mixture[b] (0.1–1.0 mg/mouse) | *In vivo* – mice (skin tumor induced by TPA) | • Inhibited inflammation with $ID_{50}$ for each substance to be: (a) Stevioside, 291.6 µg/ear, (b) Rebaudioside A, 92.2 µg/ear, (c) Rebaudioside C, 92.2 µg/ear, (d) Dulcoside A, 92.5 µg/ear, (e) Stevioside mixture, 239.9 µg/ear<br>• Stevioside prevented skin tumor formation |
| [2] | Mizushina et al. (2005) | N.R. | Stevioside[c], Isosteviol[c], Steviol[c] | *In vitro* – DNA polymerase assays<br>*In vitro* – human T-cell acute lymphoblastic leukemia cell line (MOLT-4);<br>*In vivo* – mice (ear inflammation) | • Inhibited production of DNA metabolic enzymes, human cancer cell growth and inflammatory ear edema<br>• $IC_{50}$ values of isosteviol for calf DNA polymerase $\alpha$ and human DNA polymerase $\lambda$ were 64.0 and 103 µM, respectively<br>• $LD_{50}$ value of isosteviol for MOLT-4 was 84 µM<br>• At 500 µg/ear, isosteviol reduced inflammation by 53.0 (±4.8) % (compared to TPA only group) |
| [3] | Boonkaewwan et al. (2006) | Leaves | Stevioside (1 mM), Steviol (0.1–100 µM) | *In vitro* – human monocytes cell line (THP-1), ELISA, Western blot | • Attenuated inflammatory mediators' synthesis by inhibiting I$\kappa$B$\alpha$/NF-$\kappa$B signaling pathway<br>• Stevioside suppressed LPS-induced release of TNF-$\alpha$ and IL-1$\beta$ and nitric oxide, but not steviol<br>• Only stevioside induced TNF-$\alpha$, IL-1$\beta$, and nitric oxide release in unstimulated THP-1 cells |
| [4] | Bunprajun et al. (2012) | N.R. | Stevioside (10 mg/kg) | *In vivo* – rat (muscles) | • Enhanced satellite cell activation by inhibiting NF-$\kappa$B signaling pathway<br>• Reduced NF-$\kappa$B nuclear translocation |
| [5] | Fengyang et al. (2012) | N.S. | Stevioside (50, 100 and 200 µg/ml) | *In vitro* – human macrophage cell lines (RAW264.7), ELISA, qRT-PCR, western blot | • Suppressed TNF-$\alpha$, IL-6, and IL-1$\beta$ gene expression and protein levels in LPS-stimulated RAW264.7 cells in a dose-dependent manner<br>• Reduced NF-$\kappa$B activation, I$\kappa$B$\alpha$ degradation, phosphorylation of ERK, JNK and p38 protein level |
| [6] | Boonkaewwan and Burodom (2013) | Leaves | Stevioside (0.01–1 mmol/l), Steviol (1-100 µmol/l) | *In vitro* – human colon carcinoma cell line (Caco-2), ELISA | • Attenuated cytokine gene expression via I$\kappa$B$\alpha$/NF-$\kappa$B signaling pathway<br>• Suppressed TNF-$\alpha$, IL-1$\beta$ and IL-6 release<br>• Increased I$\kappa$B$\alpha$ activation and inhibited NF-$\kappa$B expression at protein level |
| [7] | Yingkun et al. (2013) | N.R. | Stevioside (12.5, 25 and 50 mg/kg) | *In vivo* – mice (lung), ELISA, MPO and nitrate/nitrite content assay, western blot | • Attenuated histological alterations in the lung<br>• Stevioside and dexamethasone significantly decreased the number of inflammation cells.<br>• Reduced TNF-$\alpha$, IL-6, and IL-1$\beta$ protein levels<br>• Inhibited the phosphorylation of I$\kappa$B-$\alpha$ and NF-$\kappa$B<br>• Decreased protein expressions of iNOS and COX-2 |

(*continued*).

**Table 4.** Continued.

| No. | References | Plant part | Tested substance | Experimental design | Dosage and results |
|---|---|---|---|---|---|
| [8] | Wang et al. (2014a) | Leaves | Stevioside (30, 100 and 300 μg/ml) | *In vitro* – primary mouse mammary epithelial cells (MMECs), ELISA, qRT-PCR, western blot | • Suppressed the activation of the NF-$\kappa$B and MAPK pathways in *Staphylococcus aureus*-infected MMECs<br>• Decreased *TLR2* gene expression<br>• Decreased TNF-$\alpha$, IL-6, and IL-1$\beta$ gene and protein expression |
| [9] | Wang et al. (2014b) | Leaves | Stevioside (33, 100 and 300 mg/kg) | *In vivo* – mice (mammary glands), ELISA, qRT-PCR, western blot | • Inhibited inflammation by regulating the NF-$\kappa$B and MAPK pathways in *Staphylococcus aureus*-infected mouse mammary glands<br>• Suppressed the NF-$\kappa$B pathway by prominently inhibiting the phosphorylation of I$\kappa$B$\alpha$ and p65 protein<br>• Inhibited the increase of phosphorylated p38, ERK and JNK.<br>• Reduced expression of TNF-$\alpha$, IL-1$\beta$, and IL-6 at gene and protein levels<br>• Suppressed TLR2 mRNA levels |
| [10] | Holvoet et al. (2015) | Leaves | Stevioside (10 mg/kg), Rebaudioside A (12 mg/kg), Steviol (5 mg/kg) | *In vivo* – mice (liver)[d], qRT-PCR | • Attenuated hepatic steatosis extent by effecting glucose and lipid metabolism, inflammation and oxidative stress<br>• Decreased the gene expression of NF-$\kappa$B |
| [11] | Latha et al. (2017) | Leaves | Hydroalcoholic extract of stevia (*in vivo* – 500 mg/kg), Stevioside (*in vivo* – 250 mg/kg) | *In vitro* – DPPH, nitric oxide radical scavenging activity, superoxide radical scavenging activity;<br>*In vivo* – rat (liver), ELISA | • $IC_{50}$ values of stevioside and hydroalcoholic extract of stevia for DPPH assay were found to be 156.8 and 138.33 μg/ml, respectively<br>• $IC_{50}$ values of stevioside and hydroalcoholic extract of stevia for nitric oxide radical scavenging assay were found to be 102.55, 78.71 μg/ml, respectively<br>• $IC_{50}$ values of stevioside and hydroalcoholic extract of stevia for superoxide radical scavenging assay were found to be 134.6, 107.72 μg/ml, respectively<br>• Reduced hepatic oxidative stress and attenuated structural changes<br>• Reduced TNF-$\alpha$, IL-1$\beta$ and IL-6 release<br>• Increased protein levels of superoxide dismutase and glutathione in LPS-induced rats |
| [12] | Ramos-Tovar et al. (2018a) | Leaves | Aqueous extract of stevia (100 mg/kg) | *In vivo* – rat (liver), ELISA, IHC | • Ameliorated oxidative stress, necrosis as well as cholestasis and preserved hepatic architecture against TAA-induced cirrhosis<br>• Prevented liver inflammation by downregulating NF-$\kappa$B expression and partially preserved protein expression of Nrf2<br>• Reduced protein expression of TNF-$\alpha$, IL1-$\beta$, IL-6, IL-10 |
| [13] | Ramos-Tovar et al. (2018b) | Leaves | Stevia mixture[e] (100 mg/kg) | *In vivo* – rat (liver), IHC, western blot, qRT-PCR | • Prevented acute carbon tetrachloride-induced glutathione depletion and lipid peroxidation<br>• Prevented necrosis and inflammation via decreasing expression of NF-$\kappa$B and pro-inflammatory cytokines (TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-10) |

(*continued*).

**Table 4.** Continued.

| No. | References | Plant part | Tested substance | Experimental design | Dosage and results |
|---|---|---|---|---|---|
| [14] | Meng et al. (2018) | N.R. | Stevioside (*in vitro* – 200 µM; *in vivo* –10, 30 mg/kg) | *In vitro* – bone marrow-derived macrophages (BMMs); *In vivo* – mice | • Suppressed titanium particle-induced NF-$\kappa$B and MAPK signaling pathways through inhibition of TAK1 phosphorylation<br>• Inhibited production of nitric oxide and prostaglandin E2 stimulated by titanium particles<br>• Reduced the mRNA and protein expression of TNF-$\alpha$, IL-6 and IL-1$\beta$ |
| [15] | Casas-Grajales et al. (2019a) | N.S. | Stevioside (*in vitro* – 5, 10, 20 and 40 mM; *in vivo* – 20 mg/kg) | *In vitro* – Human HSCs, western blot, qRT-PCR, DPPH; *In vivo* – rats (liver and blood), IHC, western blot, qRT-PCR, DPPH | • Preserved body weight, liver/body weight ratio, glycogen content; ameliorated oxidative stress against TAA-induced cirrhosis.<br>• Prevented inflammation by reducing NF-$\kappa$B and pro-inflammatory cytokines (IL-17a, TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-10) at both gene and protein levels<br>• Improved the cellular redox state by preserving Nrf2 expression |
| [16] | Casas-Grajales et al. (2019b) | N.R. | Rebaudioside A (*in vitro* – 1, 5, 10, 20 and 100 mM; *in vivo* – 20 mg/kg) | *In vitro* – Human HSCs, western blot, qRT-PCR, DPPH; *In vivo* – rats (liver and blood), IHC, western blot, qRT-PCR, DPPH | • Prevented fibrogenesis, morphological, histological and biochemical changes against TAA-induced cirrhosis<br>• Ameliorated oxidative stress by preserving protein expression of Nrf2<br>• Prevented liver inflammation by downregulating NF-$\kappa$B and pro-inflammatory cytokines (TNF-$\alpha$, IL-1$\beta$, IL-6)<br>• Reduced expression of $\alpha$-SMA, TGF-$\beta$1, Smad7 and MMP-13 protein |

[a]Varied doses of steviol glycosides (0.008, 0.04, 0.2 or 1.0 mg/ear) were used to study anti-inflammatory activity against TPA-induced inflammation.
[b]Stevioside mixture contains stevioside (48.9%), rebaudioside A (24.4%), rebaudioside C (9.8%), dulcoside A (5.6%) and unidentified components (11.3%).
[c]Two different doses of compounds were used at 250 or 500 µg/ear.
[d]ob/ob and LDLR-double deficient mice was used for the study.
[e]Authors used commercially available stevia which is procured as Mayan Sweet Stevia® (Yucatan, Mexico).

by activating nuclear factor erythroid 2-related factor 2 (Nrf2) response, an important transcription factor that can avert inflammation. Similar findings were reported by a recent study by Casas-Grajales in 2019, in which they demonstrated that rebaudioside A preserved expression of Nrf2 as well as downregulated/prevented the expression of pro-inflammatory pathway and genes such as NF-$\kappa$B, TGF-$\beta$1, Smad7 and MMP-13 protein (Casas-Grajales et al. 2019a, 2019b).

The idea of crosstalk between Nrf2 antioxidant response and NF-$\kappa$B-inflammatory response has been proposed few years back (Li et al. 2008; Ma 2013). It has been speculated that the activators of Nrf2 can hinder IKK/I$\kappa$B phosphorylation and p65 NF-$\kappa$B subunit nuclear translocation, and then preventing NF-$\kappa$B signaling pathway. Based on the proposed theory, stevioside may possibly prevent inflammation (a) via direct actions on NF-$\kappa$B signaling pathway which then inhibiting the production of pro-inflammatory cytokines, and/or (b) via activating Nrf2 antioxidant response which then inhibits NF-$\kappa$B signaling pathway (Bunprajun et al. 2012; Yingkun et al. 2013; Garcia-Arroyo et al. 2016; Ramos-Tovar et al. 2018a, 2018b; Casas-Grajales et al. 2019a, 2019b).

## Potential development of stevioside as chemopreventive agent

Over these years, there has been substantial evidence to support the notion that chronic inflammation can increase cancer risk (Shacter and Weitzman 2002). Given that the conventional cancer treatment regime like chemotherapy often come with unwanted side effects (e.g. alopecia, mouth ulceration, nausea and vomiting), researchers are yet to design or find a 'perfect cure' that has got high drug specificity, targeting only cancer cells but not normal cells (Chabner and Roberts 2005; Chari 2007). As a hallmark feature of cancer, inflammation plays an important role in carcinogenesis – from the formation and development

of cancer cells (i.e. initiation, progression) to migration to other sites (i.e. metastasis) (Germano et al. 2008; Grivennikov et al. 2010; Hanahan and Weinberg 2011; Arvelo et al. 2016). The anti-cancer and antioxidant potential of stevia and its chemical constituents have been suggested by researchers for more than 10 years (Konoshima and Takasaki 2002; Yasukawa et al. 2002; Mizushina et al. 2005; Masuda et al. 2006; Bhattacharyya et al. 2009; Chen et al. 2018). As a high intake of antioxidants can prevent the accumulation of free radicals or oxidative stress generated by injury or even infections, they can essentially decrease cancer risk. Besides diterpenoids like austroinulin and 6-*O*-acetyl-austroinulin, stevioside as the major component of stevia plant can scavenge free radicals and eliminate cancer cells by altering pathways pivotal for their survival (Paul et al. 2012; Cho et al. 2013; Yildiz-Ozturk et al. 2015). Additionally, some researchers pointed out that rather than just reducing the accumulation of free radicals alone, it may be important to target both oxidative stress and inflammatory processes together to ensure a fruitful therapeutic outcome (Biswas 2016; Pillon Barcelos et al. 2017). In the case of stevia plant and its metabolites, researchers described their antioxidant activities based on both biochemical and cell-based assays, reducing the accumulation of free radicals by activating antioxidant pathways that drive expression of enzymes such as superoxide dismutase and catalase (Rao et al. 2014; Bender et al. 2015; Prata et al. 2017). Based on these studies, steviol glycosides have been highlighted to have a significantly beneficial role in human health maintenance. That being said, it is truly fascinating that on top of antioxidant activity, stevioside can modulate immune responses and influence recruitment of immune cells, which may be beneficial to ensure the elimination of cancer cells (Boonkaewwan et al. 2006; Arango Duque and Descoteaux 2014). Boonkaewwan et al. (2006) reported that stevioside suppressed TNF-$\alpha$, IL-1$\beta$ and nitric oxide release in monocytes after stimulated with lipopolysaccharide (LPS), whereas those unstimulated monocytes treated with stevioside induced a higher level of TNF-$\alpha$, IL-1$\beta$ and nitric oxide. Therefore, the consumption of stevioside may be useful to enhance innate immunity, particularly in augmenting macrophage function (e.g. phagocytic activity) to ensure the eradication of cancer cells (Sehar et al. 2008). Taken altogether, stevioside represents an impressive drug candidate which deserves further investigations as the compound can inhibit oxidative stress while modulating immune responses, both processes which are said to be critical players in the development of human chronic diseases.

## Future recommendations and direction

Reflecting on the past, it has been over a century since the discovery of *S. rebaudiana*. Yet, researchers are still trying to study the chemical constituents of the stevia and make use of every molecules that is isolated from the plant. Stevioside has always been in the limelight, owing to its commercial importance as a natural sweetener. Even so, growing evidence reveals that stevioside may possibly be developed as a valuable pharmaceutical agent against inflammation, given that the compound itself does not exhibit any cytotoxicity (Curry and Roberts 2008; Tandel 2011). Hence, there have been much efforts that has been poured in to 'improvise' its chemical structure, in the hope of exploiting it for medicinal purposes. Through acid hydrolysis, stevioside is converted into another beyerane diterpene known as isosteviol. Isosteviol has been shown to possess bioactivities such as antihyperglycemic (Ma et al. 2007), anti-inflammatory (Yasukawa et al. 2002) and anti-cancer effects (Mizushina et al. 2005). However, one of the major flaws of isosteviol is that it has poor water solubility, rendering the drug formulation process complicated. Thus, a research group has successfully generated isosteviol sodium (STVNa) with higher water solubility by adding a sodium group to the lead structure isosteviol, without jeopardizing its bioactive potentials (Sun et al. 2018). Similar action as stevioside, STVNa was shown to be able to regulate NF-$\kappa$B signaling pathway and transforming growth factor (TGF)-$\beta$-signaling pathways facilitated by cylindromatosis (CYLD) protein (Lim et al. 2012; Tang et al. 2018; Zhang et al. 2018). For instance, STVNa (8 mg/kg) suppressed oxidative stress and inflammation in the heart of diabetic rodent model (induced by streptozotocin), via inhibitory actions on MAPK and NF-$\kappa$B pathway (Tang et al. 2018). All in all, these findings showed that the derivatization of stevioside and its associated metabolites might be worthwhile for the search of bioactive compounds with potent anti-inflammatory actions.

Despite that, microbial biotransformation may also be another powerful manufacturing tool in the chemical and pharmaceutical industries (Xu et al. 2007;

Parshikov et al. 2012; Hegazy et al. 2015). The idea is that, through microbial biotransformation, some naturally occurring inactive compounds may occasionally be transformed into its active form by microbes (Musharraf et al. 2010; Parshikov et al. 2012). As a green chemistry, this biotransformation method complements with the Sustainability Development Goals proposed by the United Nations (https://www.un.org/sustainabledevelopment/), redu cing energy consumption and minimizing and/or eliminating the use of hazardous substances. Akihisa et al. (2004) demonstrated that fungus can be used to modify isosteviol structure, yielding different or new compounds based on the fungus used (e.g. *Aspergillus niger*, *Glomerella cingulate*, *Mortierella elongate*). Using Raji cell models to study the activation of Epstein–Barr virus early antigen, five of the products generated from biotransformation exhibited more potent inhibitory effects (92–96% inhibition rate) than isosteviol and stevioside, while preserving high viability (60%) of Raji cells. Complementary with the use of microbes to generate next-generation sweeteners, this biotransformation process may improve the taste of stevia by diminishing the lingering bitter taste, and simultaneously increase its bioactive potentials for pharmaceutical purposes (Olsson et al. 2016).

Along with that, several groups have decided to take a closer look at the mechanisms of anti-inflammatory activity conferred by stevia and steviol glycosides, focusing on the changes in intestinal microbiome following the intake of this natural sugar substitute. Without displaying any obvious toxicity to humans, some studies hinted that the intake of non-caloric sweeteners can affect the microbial population in the gut (Suez et al. 2014, 2015; Bian et al. 2017; Wang et al. 2018; Nettleton et al. 2019; Vamanu et al. 2019). Instead of being absorbed into the bloodstream, these sweeteners may accumulate in the lumen of the gut, causing dysbiosis or imbalance in the gut microbiome. Stevia extract containing steviol glycosides is able to kill pathogens like *Escherichia coli* O157:H7, but not probiotics *Bifidobacterium* and *Lactobacillus* (Tomita et al. 1997). In gastrointestinal diseases like inflammatory bowel disease, stevioside has been shown to be capable of ameliorating inflammatory symptoms, while preserving histo-architecture of the mice colon (Alavala et al. 2019), which could be attributed to the restoration of the gut microbiome or via regulation of their metabolites (Atteh et al. 2008). In spite of that, a study in Romania by Vamanu et al. (2019) investigated the changes in microbiome and metabolomics after exposed several sweeteners using an *in vitro* static system (GIS1 model), including three forms of commercially purchased steviol (i.e. powder, tablet or combination with brown sugar). The team noted mixed findings; even though the total number of bacterial cells did not change, steviol capsules significantly reduced Gram-negative strains and *Bifidobacteria*, while steviol powder and steviol containing brown sugar increased the number of *Bifidobacteria*. Undeniably, the authors highlighted the probable interference by carrier ingredients such as sodium bicarbonate. These results then urge further research to unravel potential anti-inflammatory action of stevia acting via regulation of gut microbiota.

Collectively, there have been much research work revolving identification of chemical constituents in stevia plant, as well as exploring their potential as a natural sweetener. Lest we ignore that as a natural source of anti-inflammatory products with low toxicity, the continuous efforts on stevia studies prove that it is more than just a sweetener and warrants it for further investigation to be developed as valuable pharmaceutical or therapeutic agents against important human diseases like cancer.

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No potential conflict of interest was reported by the authors.

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## Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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