REVIEW

The Potential Use of Stevia (*Stevia rebaudiana*) in Coronavirus Diseases (COVID-19)

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Disclosure

The authors of this review have no competing interest in this subject.

Disclaimer

This review is essentially a brief report, prepared on an urgent basis, to reflect the highest level of evidence available regarding the subject at this specific time. The conclusion draws on restricted reviews from analysis of pertinent literature, on expert opinion and/or regulatory status where appropriate. All efforts have been made to ensure all relevant published material has been reviewed but this document may still not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of this review.

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Executive Summary

Title

The Potential Use of Stevia (*Stevia rebaudiana*) in Coronavirus Disease (COVID-19)

Objective

The objective of this report is to assess current available evidence on the potential of *Stevia rebaudiana* in COVID-19 management based on the following:

- Efficacy: Focus on 1: antiviral properties of stevia extract and 2: the steviol glycosides potential in the modulation of immune response including anti-inflammatory, and role as other supportive therapy or management of COVID-19; and their respective potential mechanism(s) of actions.
- Safety on stevia extract, steviol and steviol glycosides (stevioside and rebaudioside A).

Methodology

Electronic databases were searched using pre-determined terminologies such as as '*Stevia rebaudiana*', 'Stevia', 'steviol glycosides', 'antiviral', immunomodulatory', 'immune response', 'inflammation', 'mechanism of action', and 'safety'. Any peer-reviewed journals found were included, which covers PubMed, Ovid Medline®, EBM Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Cochrane database of systematic reviews. The articles included in the search strategy were limited to those which were published within the year 1991 to November 2019. All clinical and preclinical studies (both in vitro and in vivo) related to safety and efficacy or effectiveness of *S. rebaudiana* extract and its main chemical constituents were included.

Results & Discussion

- There is no documented evidence of the antiviral effects of *Stevia rebaudiana* and steviol glycosides for treatment of COVID-19 or any viral and respiratory infection.
- One preliminary study highlighted the antiviral activities of purified stevia extracts against coronavirus, bovine herpes virus and porcine teschovirus. Nevertheless, the effective concentration of the extract used was exceedingly high. This study is lacking in cytotoxicity data and the purity of the steviol glycosides purified from the extract was also not mentioned.
- Stevia leaf extracts have also been reported to inhibit the growth of human rotavirus and herpes simplex virus type 1. In these studies, the polysaccharides are reported to inhibit the virus entry into the host cells.

- Although limited literature is available, anti-inflammatory roles of stevioside have been observed using in vitro and in vivo models.
- Cytotoxicity study on various cell lines indicated no toxicity for up to ~2,000 µM of stevioside and rebaudiosides A, and 100 µM of steviol.
- Based on the safety documents, high purity stevia extracts (at least 95% steviol glycosides) are generally considered to be safe for human consumption at a human equivalent dose of 4 mg/kg body weight/day. The findings of toxicity studies showed that steviol glycosides (stevioside and reb A) are not genotoxic, carcinogenic or associated with any toxicity for reproduction/developmental and are unlikely to produce adverse effects when tested on animal models and cell (in vitro and in vivo) in short and long term administration. On the other hand, although steviol and some of its oxidative derivatives have been shown in one study to produce in-vitro genotoxicity, this was not expressed in vivo. Indeed, the Joint Expert Committee for Food Additives (JECFA) report concluded that this issue is addressed by the fact that steviol is present if any at a negligible level in the systemic circulation in humans.

Conclusion

Considering the present safety profile of stevia (*Stevia rebaudiana*) extract, their potential as antiviral in the treatment of COVID-19 may be explored in future investigations. However, this should also include the polysaccharides (usually obtained from water or ethanolic extract) and not only limited to the steviol glycosides. The steviol glycosides (stevioside, steviol and Reb A) were reported non-toxic on several cells, which ranged from 100 to 2,000 µM. However, the current data do not indicate the efficacious dose. Therefore, it is suggested that a pilot study is performed to determine the half maximal cytotoxic concentration (CC_{50}) and half maximal effective concentration (EC_{50}) of these compounds against the normal cell line and the virus, respectively. These values are needed for determination of selectivity index (SI) or specificity level of compound activity against the viral growth. From this pilot study, further studies will only be recommended if the anti-SARS-CoV-2 activity of a compound is greater than the cytotoxic activity on normal healthy cells or host cells (SI ≥ 10).

Full report on stevia (*Stevia rebaudiana*) potential in COVID-19 management

Introduction

Stevia products have been authorized as a food additive in many countries. Stevia contained primarily steviol glycosides extracted and purified from *Stevia rebaudiana* Bertoni leaves [1]. Steviol glycosides are a group of compounds, which are responsible for the sweet taste of Stevia leaves. At least ten types of steviol glycosides (stevioside, rebaudioside A (reb A), rebaudioside B (reb B), rebaudioside C (reb C), rebaudioside D (reb D), rebaudioside E (reb E), rebaudioside F (reb F), dulcoside A, rubusoside and steviolbioside) have been reported in stevia extract [2]. However, the main steviol glycosides from the leaves extract are stevioside and reb A. Pharmacokinetic analysis has shown that the metabolic and elimination pathways of reb A and stevioside in humans are similar [3]. In human and animals, both stevioside and reb A are poorly absorbed after oral exposure but they are metabolized in the lower intestinal tract by intestinal microflora to steviol. The majority of circulatory steviol is in the form of steviol glucuronide and this metabolite is primarily excreted in the urine while steviol is excreted into feces [3]. Although stevia is not known traditionally to treat fever or infectious diseases, the company PureCircle requested to evaluate the potential of steviol glycosides against 2019-nCoV based on a preliminary study which tested the enriched extract of stevia against human coronavirus (Hco V-229E) [4].

Objective

The objective of this report is to assess the currently available evidence on the potential of stevia (*Stevia rebaudiana*) extract and its main chemical constituents (steviol glycosides and steviol) in COVID-19 management based on the following:

- Efficacy: Focus on 1: antiviral properties of stevia extract and 2: the steviol glycosides potential in the modulation of immune response including anti-inflammatory, and role as other supportive therapy or management of COVID-19; and their respective potential mechanism(s) of actions.
- Safety on stevia extract, steviol and steviol glycosides (stevioside and rebaudioside A).

Methodology

Searching

Electronic databases were searched using pre-determined terminologies such as ‘stevia rebaudiana’, ‘stevia’, ‘steviol glycosides’, ‘antiviral’, immunomodulatory’,

'immune response', 'inflammation', 'mechanism of action', and 'safety'. Any peer-reviewed journals found were included, which covers PubMed, Ovid Medline®, EBM Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Cochrane database of systematic reviews. The articles included in the search strategy were limited to those which were published within the year 1991 to November 2019. All clinical and preclinical studies (both in vitro and in vivo) related to safety and efficacy or effectiveness of *S. rebaudiana* extract and its main chemical constituents were included.

Selection

All published articles related to safety and efficacy or effectiveness of *S. rebaudiana* and steviol glycosides in treating viral diseases were included. Published articles reviewed in this report on the safety of stevioside and rebaudioside A are mainly studies which complied with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications (with at least 95% purity).

Results & Discussion

Efficacy

Antiviral effects:

- An anti-bovine herpes virus, anti-porcine teschovirus, and anti-human coronavirus assay performed on the purified extract of stevia leaves showed antiviral activity towards porcine teschovirus, human coronavirus and bovine herpes virus at very high dose of 2000-4000 µg/mL [4].

- Anti-human rotavirus in vitro assay study found that the hot water extract of *S. rebaudiana* inhibited the replication of human rotavirus. The anionic polysaccharide molecule of stevian (purified fraction) possibly interrupts with virus-host cell receptor interaction [5].

- An anti-herpes simplex virus type 1 in vitro study showed that polysaccharide, arabinogalactan obtained from stevia extract exhibited a promising anti-Herpes activity with selectivity index > 1,000 and EC_{50} value < 1 µg/mL [6].

- Other anti-herpes simplex virus type 1 in vitro study also revealed that the polysaccharide fractions (crude fraction & homogeneous alkaline fraction) of stevia leaves extract inhibited viral adsorption and penetration [7].

Immunomodulatory effects:

- An in vitro study using stevioside with purity ≥ 98% was extracted from dried *S*. *rebaudiana* leaves; 0.01-1 mM) and steviol (approximately 90% purity was obtained by further purification of the crude stevioside by oxidation; 1-100 µM) attenuates LPS-induced tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) production in colonic epithelial cells. Both substances also showed immunomodulatory effects on inhibitor of nuclear factor kappa B (IκBα) activation and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) suppression in western blotting [8].

- An in vitro study using stevioside (98% purity was extracted from dried *S*. *rebaudiana* leaves; 0.01-1 mM) and steviol (approximately 90% purity was obtained by further purification of the crude stevioside by oxidation; 1-100 µM) on human acute monocytic leukaemia cell line (THP-1) showed that stevioside at 1 mM significantly suppressed lipopolysaccharide (LPS)-induced release of tumour necrosis factor receptor (TNF-R) and interleukin 1 alpha (IL-1α) and slightly suppressed nitric oxide release in THP-1 cells without exerting any direct toxic effect, whereas steviol at 100 µM did not. Using Western blotting, activation of inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) and transcription factor NF-kB were suppressed by stevioside only [9].

- An in vitro study using stevioside with purity ≥98 % (300, 100, or 30 µg/mL) on *Staphylococcus aureus*-infected mouse mammary epithelial cells (MMECs) suppressed the *S. aureus*-induced expression of toll-like receptor 2 (TLR2) and proteins of the NF-κB and mitogen-activated protein kinase (MAPK) pathways in a dose dependent manner as well as apoptosis [10].

- An animal study using stevioside with purity ≥ 98% (300, 100, and 33 mg/kg) administered intraperitoneally for three times (6, 8 and 12 hours) after 24 hours of inducing infection in the mammary gland of lactating BALB/c mice (6–8 weeks old, weighing 40–45 g) significantly ($p < 0.05$) reduced the inflammatory cell infiltration and the levels of TNF-α, IL-1β, and IL-6 and the respective expression of their messenger RNAs (mRNAs) in a dose-dependent manner. Stevioside downregulated the TLR2, NF-κB, and (mitogen-activated protein kinase) MAPK signalling pathways in the *S. aureus*-infected mouse mammary gland [10].

Safety

Cytotoxicity:

- Stevioside and steviol were evaluated for their toxicity effect on Caco-2 cells using MTT assay. Results showed that after 12 hours of treatment, no cytotoxicity was observed at the concentration range of stevioside (1–1,000 μM) and steviol (0.1–100 μM) [8].

- Cytotoxicity of *S. rebaudiana*, stevioside and reb A extracts were evaluated by measuring the cytosolic lactate dehydrogenase activity, with the LDH cytotoxicity kit toward HepG2 cells. A dose of 1 mg/mL of each extract was administered to cells up to 22 hours. Stevia extracts at 1 mg/mL concentration showed slight to no cytotoxic effect after 22 hours of treatment. Hence, no-cytotoxic concentrations of the samples were used in the cell-based assays. LDH activity was also evaluated in HepG2 cells treated with stevioside and Reb A purified extracts, no toxicity was observed at the tested concentrations (0.5, 1 and 2 mg/mL). [11]

Acute toxicity:

- The acute toxicity of stevioside and steviol was investigated in rat, mouse and hamster by treating them intragastrically. Stevioside at a dose as high as 15 g/kg body weight was not lethal to either mice, rats or hamsters. Hamsters were found to be more susceptible to steviol than rats or mice. LD_{50} values of steviol in hamsters were 5.20 and 6.10 g/kg body weight for males and females, respectively. Histopathological examination in the kidney of hamsters induced by steviol revealed severe degeneration of the proximal tubular cells. These structural alterations were correlated with the increases in serum blood urea nitrogen (BUN) and creatinine. Therefore, the possible cause of death induced by steviol might be due to acute renal failure. [12]

Sub-acute toxicity:

- Sub-acute toxicity studies were performed on F344 male and female rats and hamsters fed with up to 2.5 g/kg body weight/day of purified stevioside for three months. Stevioside showed no adverse effect at a dosage of 2.5 g/kg/day in the tested animals. Consequently, it was concluded that the no observed effect level (NOEL) for stevioside is higher than 2.5 g/kg body weight/day and the acceptable daily intake (ADI) can be at least 25 mg/kg body weight. [13]

- In a 4-week study, Wistar rats were administered reb A at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm. The NOAEL,

including an evaluation of testes histopathology, was determined to be 100,000 ppm [14].

- Toxicity of reb A was studied at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm in Wistar rats during a 13-week study. The NOAEL was considered to be 50,000 ppm or approximately 4,161 and 4,645 mg/kg body weight/day in male and female rats, respectively, and these values are about 1000 folds higher than likely for human exposures to reb A through its use as a natural sweetener. [14]

- The effects of administration of *S. rebaudiana* extract for 20 days on renal function and mean arterial pressure in normal Wistar rats were evaluated. Results showed that the *S. rebaudiana* treated rats group for 20 days did not significantly differ from the control group. [15]

Sub-chronic toxicity:

- Reb A toxicity was evaluated by administering it as a dietary admix (a mixture of reb A and normal diet of rats) at target exposure levels of 500, 1,000, and 2,000 mg/kg body weight/day to Sprague Dawley rats for 90 days. The study demonstrated that dietary administration of high concentrations of reb A (2,000 mg/kg) for 90 consecutive days to Sprague Dawley rats was not associated with any signs of toxicity [16].

- Oral administration of *S. rebaudiana* ethanolic leaves extract (containing isochlorogenic acids), at dietary levels of 1.04%, 2.08% and 3.12% for 90 days did not induce significant behavioural, haematological, clinical, or histopathological changes in rats. Significant reduction of cholesterol, total protein and albumin levels ($p < 0.05$) was observed in female animals only at high dose level. [17]

- The oral administration of fermentative reb A to Sprague Dawley rats for at least 91 days did not lead to any adverse effects at consumption levels up to 2,057 mg/kg body weight/day for males and 2,023 mg/kg body weight/day for females, which were concluded to be the NOAEL [18].

- Administration of a crude extract of *S. rebaudiana* (66.7 g of dried leaves/100 mL final solution) in normal Wistar rats for 40 and 60 days induced hypotension, diuresis and natriuresis with glomerular filtration rate (GFR) constant. An increase in the renal plasma flow (RPF) was exclusively observed for the group treated for 60 days. The results suggested that 2 mL oral administration of 13.3 g/kg body weight of aqueous extract of stevia dried

leaves twice a day to a rat weighing 100 mg induced systemic and renal vasodilation, causing hypotension, diuresis and natriuresis. The dose of *S. rebaudiana* used in this study was too high and was much more than the acceptable daily intake for human. [15]

Reproductive toxicity:

- Reb A was administered via the diet to male and female Han Wistar rats at 0, 7,500, 12,500, and 25,000 ppm for two generations. Reb A treatment was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. The NOAEL for reproductive effects was 25,000 ppm. The NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm which corresponds to the achieved intake dose of 2,048 mg/kg body weight/day in male rats and 2,273 mg/kg body weight/day in female rats. [19]

- Teratogenicity of stevioside was evaluated in pregnant Wistar rats given different doses of 0, 250, 500 and 1,000 mg/kg body weight/day by gavage once a day, from day 6 through 15 of pregnancy. The result showed that stevioside was not teratogenic and no detectable adverse effect was observed in the pregnant rats and their fetuses. Hence, it was concluded that 1,000 mg/kg body weight/day of stevioside could not cause any fetal malformation and is safe for both pregnant rats and rat fetus. [20]

Genotoxicity:

- In vitro toxicity test using bacterial reverse mutation assay, mouse bone marrow micronucleus assay and mouse sperm malformation assay showed that *S. rebaudiana* ethanolic extract from leaves (containing isochlorogenic acids), possess no adverse effects at dosage level up to 300 times of the recommended daily intake [17].

- An in vitro study (Comet assay) with dose as high as 500 µg/mL steviol did not damage the nuclear DNA of TK6 and WTK1 cells in the presence and absence of S9 mix. The in vivo studies on mice conducted by oral administration of steviol and stevia extract at 250, 500, 1,000, and 2,000 mg/kg body weight concluded the absence of DNA-damaging activity in cultured cells and mouse organs. [21]

- In vivo micronucleus assays of steviol, at concentrations of 8,000 mg/kg body weight was administered to Swiss mouse, 8,000 mg/kg body weight to Wistar rat and 4,000 mg/kg body weight to Syrian golden hamster, did not produce any micronucleus formation [22].

- In chromosomal aberration test using Chinese hamster lung fibroblasts, steviol (99% purity) did not produce genotoxicity effects at 0.125–5 mg/mL (without S9 metabolic activation) and 0.5 mg/mL (with S9 metabolic activation). S9 fraction is prepared from the liver tissue of rats to measure the metabolism of drugs or to mimic the metabolism of test substances in in-vitro assays. However, positive effects were observed when treated at 1.0–1.5 mg/mL (with S9 metabolic activation). [23]

- Stevioside (83% purity) was not mutagenic when tested using Ames test with *S. typhimurium* TA 1535, 1537, 97, 98, 100, 102, 104 *E. coli* WP2uvrA/pkM10 strains at 0.05–5 mg/plate (without metabolic activation system) 0.05–1 mg/plate (with metabolic activation system) [23].

- Stevia (active ingredient steviol glycoside) analyzed in human peripheral blood lymphocytes using chromosomal aberrations showed no genotoxic activity at a concentration up to 16 µg/mL [24].

- A bacterial reverse mutation assay and an in vitro micronucleus test conducted with fermentative reb A provide evidence for its absence of mutagenicity, clastogenicity and aneugenicity [18].

- Reb A was investigated for its potential to induce genotoxicity in three in vitro and two in vivo assays. Reb A was non-mutagenic in an Ames test using *Salmonella typhimurium* and *Escherichia coli*, in a chromosomal aberration test using Chinese Hamster V79 cells and in a mouse lymphoma assay using L5178Y+/- cells. All studies were conducted at concentrations up to 5,000 µg/mL, with and without metabolic activation. Also, reb A was non-genotoxic in a bone marrow micronucleus test in mice at doses up 750 mg/kg body weight and an unscheduled DNA synthesis test in rats at 2,000 mg/kg body weight. These studies provide additional evidence that reb A is not genotoxic at the doses tested. [25]

Carcinogenicity:

- Long-term studies of the carcinogenic potential of stevioside in male and female Fischer 344 rats showed no carcinogenic or pre-carcinogenic activity after 104 weeks on diets of up to 5% stevioside [26].

Nephrotoxicity:

- Blood urea nitrogen (BUN) and plasma creatinine levels, along with

simultaneous ultrastructural changes of the kidney were studied in rats treated with stevioside. BUN levels increased at three hours onward after subcutaneous injection with stevioside (1.5 g/kg body weight). The maximum increases in BUN and creatinine were approximately 180% and 132% at nine hours after stevioside injection, respectively. Histopathological examination of the kidney induced by stevioside revealed degeneration of the proximal convoluted tubule cells but no relation to lipid peroxide formation was detected. These results suggest that stevioside induced nephrotoxicity at the proximal convoluted tubules rather than at the glomeruli and other tubules presumably by a defect of cell volume regulation due to depletion of intracellular ATP and disruption of microvilli, and nuclear dysfunction. [27]

Clinical studies

A clinical trial evaluated the effects of four weeks consumption of reb A on healthy volunteers with normal or low-normal blood pressure and repeated use for 16 weeks on glucose homeostasis in men and women with type 2 diabetes mellitus. Reb A was administered at three doses (500 mg, 750 mg, and 1,000 mg/kg body weight) to individuals with normal glucose tolerance (n = 45) or type 2 diabetes mellitus (n = 48). Results from this clinical trial suggest that acute consumption of up to 1,000 mg of reb A has no clinically important acute effects on glucose homeostasis or blood pressure among individuals with normal glucose tolerance or type 2 diabetes mellitus. [28]

Conclusion

Considering the present safety profile of stevia extract, their potential as antiviral in the treatment of COVID-19 may be explored in future investigations. However, this should also include the polysaccharides (usually obtained from water or ethanolic extract) and not only limited to the steviol glycosides. The steviol glycosides (stevioside, steviol and reb A) were reported non-toxic on several cells, which ranged from 100 to 2,000 µM. However, the current data do not indicate the efficacious dose. Therefore, a pilot study needs to be performed to determine the half maximal cytotoxic concentration (CC_{50}) and half maximal effective concentration (EC_{50}) of these compounds against the normal cell line and the virus, respectively. These values are needed for determination of selectivity index (SI) or specificity level of compound activity against the viral growth. From this pilot study, further studies will only be recommended if the anti-SARS-CoV-2 activity of a compound is greater than the cytotoxic activity on normal healthy cells or host cells (SI ≥ 10).

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