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Can Stevia Reduce Inflammation in COVID-19 Disease?

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Abstract

The relationship between COVID-19 and the impaired balance between radicals and antioxidants is discussed. Stevia and steviol glycosides are known natural sweeteners. However, in large amounts they also possess several beneficial effects on diseases in which radicals are involved. Their radical scavenging activity in 5 assays is discussed, as well as their reported immune responses. As there is no targeted COVID-19 therapy available, Stevia crude extracts and steviol glycosides are suggested to treat COVID-19 patients. Due to the proven presence of steviol glucuronide in the blood after consuming Stevia or steviol glycosides, their radical scavenging in different organs of the human body is plausible, the more as the steviol glucuronide level in the blood is sufficiently high (± 5 μM to 20 μM) and can be increased by doubling the intake. Crude Stevia leaf extract might still be better, as they contain, besides the steviol glycosides, minerals, vitamins, a huge number of polyphenols, flavonoids and other scavenging molecules. The Stevia products can be administered without problems because the suggested daily amounts (3 mg × 250 mg stevioside) are without health problems as they are similar to the ADI for steviol glycosides. It is also generally accepted that consumption of 5 g dried Stevia leaves is without health problems.

Keywords: Covid-19; Sepsis; Crude stevia extracts; Steviol glycosides

Introduction

Since December 2019, the world population is hit by a new corona virus named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causing acute atypical respiratory diseases with a huge number of deaths world-wide. The WHO declared the Coronavirus Disease 19 (COVID-19) a pandemic. Momentarily, there is no targeted therapy available [1]. Several factors do influence the severity of the disease as there is obesity (BMI above 30), hypertension, cardiovascular disease and lung disease [1-3]. These diseases are provoked or related to an imbalance between radicals and antioxidants (enzymatic and non-enzymatic, e.g., Vit C, GSH). The appearance of COVID-19 in China was correlated to a selenium deficiency in the population of the Hubei Province of which Wuhan is the capital. This Se deficiency also increased the virulence of RNA viruses such as influenza A and coxsackievirus B3, which is the viral cofactor of the Keshan disease (cardiomyopathie). Se Supplementation drastically decreased the Keshan disease. A decreased amount of selenoproteins which regulate Reactive Oxygen Species (ROS) might well be associated with the severity of COVID-19 development [4]. It is known that Se is a potent nutritional antioxidant that carries out biological effects through incorporation in selenoproteins, which regulate ROS, cell signaling and redox homeostasis in nearly all tissues. Dietary Se influences inflammation and different immune responses in different ways. Selenoproteins are important antioxidant defense systems maintaining redox homeostasis, which also includes Catalase (CAT), Superoxide Dismutase (SOD), Glutathione (GSH), Vitamin E and C, and carotenoids [5,6]. It was shown that an optimal nutritional status is required for a well-

functioning immune system to protect against viral infections. Vitamins, including Vit A, B6, B12, C, D, E and folate, minerals like zinc, iron, selenium, magnesium and copper and omega-3 fatty acids play important and complementary roles in the support of the immune system [7].

Vitamin C infusion (50 mg/kg for 96 h) had no significant effect on organ failure or biomarkers of inflammation and vascular injury in patients with advanced stages of sepsis and Severe Acute Respiratory Failure (ARDS) [8]. A reported significant reduction of 28-day all-cause mortality should be considered exploratory as further studies are required. A high intravenous dose of Vit C is suggested for the prevention and treatment of COVID-19 [9] as well as Vit D supplementation [10]. Nutraceuticals might also have the potential for boosting the type 1 interferon response to RNA viruses including influenza and corona virus and reducing the oxidative status [11].

As no targeted COVID-19 therapy is available and the disease is related to an imbalance of radicals and anti-oxidants, it might be successful to evaluate the effects of steviol glycosides and certainly of crude Stevia extracts as these have a radical scavenging activity far better than that of Vitamin C [12,13].

What is stevia and what are steviol glycosides?

Let us first consider some definitions. What is meant by "Stevia-crude extracts-steviol glycosides-modified steviol glycosides, steviol glucuronide"?

Stevia rebaudiana (Bert.) Bertoni is an herb from Southern America that is grown now all over the world. Its leaves contain sweeteners called steviol glycosides. The sweetening properties as well as the technical aspects of extraction, purification and dosage of Stevia and steviol glycosides have been well documented [14-18]. In this text, the word Stevia is used to refer to the living plants or its dried leaves. An excellent overview of the botany, its constituents, phytochemistry, synthetic investigations, methods to improve the taste of the sweeteners and use of the sweeteners in Japan and Korea has appeared [19].

Steviol glycosides: The purified sweeteners of Stevia leaves. In some countries, the mixture of steviol glycosides is called "steviosides". However, this term is confusing and should be avoided as stevioside is only one specific compound of the mixture. An overview of the occurrence, biosynthesis and distribution of the different steviol glycosides of Stevia has been published [20].

Purity of steviol glycosides: The purity of steviol glycosides (comprising the most abundant sweeteners present, stevioside and rebaudioside A is defined as the sum of all steviol glycosides present in a mixture and expressed on a dry weight basis. A (required) purity of ≥ 95% means that the sum of the steviol glycosides makes up at least 95% of the dry weight of a sample.

Steviol Equivalents: The sweeteners have different molecular weights, and are degraded to steviol by the bacteria of the colon. Therefore, JECFA proposed to use the term "steviol equivalents" to propose an ADI of 0 mg to 4 mg steviol equivalents/kg body weight, i.e., 10 mg stevioside or 12 mg rebaudioside A/kg body weight, respectively.

Crude Stevia Extracts: The unpurified water or alcoholic leaf extracts. They are sold as Stevia syrups or powders. Their color is dark brown. The composition of the crude extracts is very complex and besides minerals, vitamins and steviol glycosides, other glycosides are found as well as different labdanes, triterpenoids and their glycosides, flavonoids, sesquiterpene lactones and polyphenols [21,22]. Although a huge number of compounds have been identified and antioxidant activity is claimed (often only in the DPPH assay), often no positive controls were included making inter-laboratory comparison very difficult the more as this assay suffers from yellow pigments present in crude extracts [12]. Moreover, no ADME studies with isolated compounds were done. Therefore, it is difficult to identify the compounds with antioxidant activity in the body. An ADME study will avoid myths about beneficial effects of antioxidants as happened, e.g., for polyphenols in red wine.

Modified Steviol Glycosides: Enzymatically or chemically modified steviol glycosides are those glycosides to which extra sugar units were attached by enzymes, GMO's or a chemical reaction. The taste profile of these mixtures of compounds is very good. However, their sweetness is only about 100 × that of a 0.4% sucrose solution, whereas that of unmodified steviol glycosides is about 250 times to 350 times sweeter.

Steviol Glucuronide: After oral intake, steviol glycosides are not absorbed by the intestines, but degraded by bacteria of the colon. Thereafter, the free steviol is absorbed and transformed into steviol glucuronide in the liver. This compound migrates to the peripheral blood and is completely excreted in the urine by the kidneys [23,24]. Steviol glucuronide is probably the active principle provoking all the pharmacologic effects.

Pharmacologic Effects

A clear distinction should be made between small doses of steviol glycosides used for sweetening purposes (estimated intake around 250 mg/day to 300 mg/day), and high doses in which case beneficial pharmacologic effects might occur. However, the high doses (750 mg/day to 1500 mg/day) having pharmacologic effects will probably be reached only after intake of capsules or tablets with pure stevioside, e.g. 3 times daily 250 mg. The pharmacologic effects reported have been obtained with stevioside or mixtures of steviol glycosides in most cases with a large proportion of stevioside. High doses of steviol glycosides improve blood pressure, type 2 diabetes and immunology. There are also anti-carcinogenic effects and preventive effects on the development of atherosclerosis [14,25]. In a mouse model for obesity, stevioside treatment improved insulin Signaling and Antioxidant Defense (SOD, CAT) in both adipose tissue and the vascular wall, leading to an inhibition of atherosclerotic plaque development and plaque stabilization. In a mouse model for obesity and insulin resistance, it was shown that stevioside, rebaudioside A and steviol attenuated liver steatosis, although there was no influence on the weight of the animals [26].

Steviol glucuronide was suggested as the active principle provoking the pharmacologic effects of high doses. Most of the pharmacologic effects observed are related to or may be explained by the radical scavenging activity of stevioside and steviol glucuronide, and/or the synthesis of enzymes destroying radicals like SOD and CAT. The effects of other compounds influencing the above processes might also be explained due to their radical scavenging activity. Crude Stevia extracts were also very strong and much better radical scavengers and had also beneficial healing effects on the above cited processes [12-14].

It is known that steviol glycosides are not absorbed by the intestines [27,28]. They are degraded by the bacteria of the colon into steviol, which is easily absorbed and transformed in the liver into steviol glucuronide. This steviol glucuronide can be found in the peripheral blood and it is filtered out by the kidneys and excreted in the urine [23,29]. Steviol glucuronide was the only compound found in the blood and was suggested as the active principle provoking some pharmacologic effects when stevioside is administered in large oral amounts of 750 mg/d up to 1500 mg/d [23,24]. Indeed, recently it was shown that steviol glucuronide stimulates insulin secretion in a dose-and glucose-dependent way from mouse islets of Langerhans [30].

The early reports on pharmacologic effects of crude Stevia extracts have been sufficiently documented [14,31]. Aqueous Stevia leaf extracts had an anti-diabetic activity in STZ-induced diabetic mice in a dose-dependent way (between 1.8 and 8.6 mg extract/kg bw). Blood glucose and the level of LDL decreased whereas the HDL significantly increased [32]. Crude ethanol extracts of Stevia leaves given orally (between 200 and 400 mg/kg bw) showed a significant reduction in blood glucose levels in alloxan-induced diabetic rats [33].

Immunologic Effects

The immune system constitutes the host defense against invading pathogens, foreign components and cancer cells. Inflammatory processes, including the release of pro-inflammatory cytokines and formation of Reactive Oxygen (ROS) and Reactive Nitrogen Species (RNS), are an essential part of the immune responses. Although these actions are usually followed by an anti-inflammatory response, excessive production of pro-inflammatory cytokines may lead to chronic inflammation. This is the situation found in patients with severe Corona disease. Pathogenic bacteria and other infectious agents like viruses can activate monocytes or macrophages directly, initiating a cytokine cascade in the inflammatory process and the immunological response. Stimulated monocytes release a broad spectrum of cytokines, such as the biologically active peptides Tumour Necrosis Factor-α (TNF- α) and Interleukin-1β (IL-1β) and IL-6. In addition, the reactive free radical, Nitric Oxide (NO) also plays a role in inflammation.

From in vitro assays, some authors concluded that stevioside might have a beneficial effect on innate immunity by inhibition of the secretion of TNF-α, IL-6 and IL-1β [34-36]. It was concluded that stevioside induces TNF-α, IL-1β and NO production in non-stimulated human monocytic THP-1 cells, augmenting macrophage function and thus contributing to the enhancement of innate immunity. On the other hand, inhibition of TNF-α, IL-1β and NO release in the LPS-stimulated THP-1 cells by stevioside could be of benefit in circumstances where there is a pathological effect resulting from excess of TNF-α, IL-1β and NO productions. This action demonstrates an anti-inflammatory effect of stevioside. The consumption of stevioside may enhance the innate immunity and protect against inflammatory diseases.

In some *in vitro* assays stevioside was not active, but steviol was. The difference might be due to the size of the stevioside molecule that is too large to be absorbed by cells, as has been shown for Caco-2 Cells [28]. Steviol at the concentration of 1-100 μM inhibited the release of TNF-α and IL-1β in a dose dependent manner in lipopolysaccharide-treated human CD14+ cells. An IL-6 inhibition was found at 10 μM and 100 μM. Steviol (when consumed orally in vivo probably steviol glucuronide), possesses an anti-inflammatory activity [37].

In *in vivo* experiments with rats, the presence of inflammation was studied in metabolic disorders including Diabetes Mellitus (DM). The pro-inflammatory cytokines, IL-1β, IL-6 and TNF- α have been shown to be elevated in type1 and type2 DM [36]. Peripheral Blood Mononuclear Cells (PBMCs) are blood cells with a round nucleus, such as a lymphocyte or a monocyte. These blood cells are a critical component in the immune system. TNF-α is not usually detectable in healthy individuals. Its elevated plasma and tissue levels are found mostly in inflammatory and infectious conditions.

Rats, orally fed with 500 mg and 1000 mg stevioside/kg bw/day did not have any effect on plasma TNF-α. This result indicated that oral ingestion of stevioside did not induce any inflammation. PBMCs isolated from rats treated with 500 and 1000 mg/kg bw/day showed a reduction in TNF- α release from LPS-stimulated PBMCs [38]. The effect of oral stevioside (500 and 1000 mg/kg BW/day) was also studied in male Wistar rats. Plasma levels of TNF-α and IL-1β was not detected in control and stevioside treated groups. The release of TNF-α from LPS stimulated Peripheral Blood Mononuclear Cells (PBMCs) was significantly lower in the stevioside-treated group. The IL-1β levels in stevioside treated rats were significantly lower than in the control [39].

An immunomodulatory activity of stevioside (purity unknown) in mice was also reported [40]. At 12.5 mg/kg bw, stevioside stimulated phagocytic functions as indicated by an increased phagocytic index in a carbon clearance test, and increased humoral response, measured by an increase in antibody titre to a test antigen. *In vitro* experiments demonstrated stimulatory effects on phagocytic activity and on B and T cell proliferation stimulated by lipopolysaccharide and concanavalin A, respectively. However, more work is required to corroborate these observations.

Radical Scavenging Effects

Reactive Oxygen Species (ROS) exist as a result of the occurrence of molecular oxygen in the atmosphere. In many reactions, ROS are formed, e.g., in organelles with a high metabolic activity like mitochondria (respiration), microbodies and chloroplasts (photosynthesis, typical for plants). Organisms have to deal with these ROS and several mechanisms have been developed to keep these ROS in balance. Nitric Oxide (NO) is also an important cellular signaling molecule in many physiological and pathological processes and it is formed by Nitric Oxide Synthase Enzyme (NOS) [41].

As the above-mentioned pharmacologic effects are induced by or related to reactive oxygen and nitrogen species, we studied the possible ROS and RNS scavenging activity of steviol glycosides, steviol glucuronide and crude Stevia extracts. Some other compounds (metformin, aspirin, hydroxytyrosol) were also included in the study as they have also an effect on the above pharmacologic effects. Vitamin C and quercetine were used as a positive control. The following radicals were studied: DPPH, hydroxyl radicals, superoxide, NO and TBA reactive material. To be able to compare the activity of various compounds, the IC_{50} values are given in mM (concentration inhibiting 50% of the radicals formed) [12,13].

Table 1 shows that the positive control quercetine was the most active •OH scavenger (IC_{50}: 0.115 mM), followed by the group of steviol glycosides (IC_{50}: 0.22 mM) and steviol glucuronide (IC_{50}: 0.21 mM). Also, aspirin had a good radical scavenging activity (IC_{50}: 0.305 mM) whereas metformin and hydroxytyrosol scored about the same as ascorbic acid (IC_{50}: 1.154 mM). In superoxide scavenging, ascorbic acid was most active (IC_{50}: 0.06 mM), followed by steviol glucuronide (IC_{50}: 0.211 mM), quercetine (IC_{50}: 0.32 mM) and hydroxytyrosol (IC_{50}: 0.51 mM). Steviol glycosides were less efficient scavengers than steviol glucuronide (IC_{50}: >1.4 mM). Quercetine was very potent in reducing the TBA reactive material (IC_{50}: 0.912 mM), followed by hydroxytyrosol (IC_{50}: 2.34 mM), ascorbic acid (IC_{50}: 11.3 mM) and metformin (IC_{50}: 104 mM). The value for steviol glucuronide activity was intermediate (IC_{50}: 149 mM). Steviol glycosides were less efficient than steviol glucuronide (IC_{50}: > 300 mM). Only the positive controls and hydroxytyrosol could scavenge DPPH (IC_{50}: 0.055 mM for ascorbic acid; IC_{50}: 0.186 mM for hydroxytyrosol; IC50: 13.8 mM for quercetine) and NO radicals (IC_{50}: 0.015 mM for ascorbic acid; IC_{50}: 0.184 mM for quercitine; IC_{50}: 34.3 mM for hydroxytyrosol). All the other tested compounds were without activity.

Crude plant extracts were very potent ROS and RNS scavengers in all assays used and virtually destroyed all radicals. IC_{50} values could not be calculated as the extracts were mixtures of a huge number of (unknown) compounds. Part of the scavenging activity of crude plant extracts was due to phenols or polyphenols that could be removed by PVPP treatment. Most of the residual scavenging activity remaining after PVPP treatment could be removed by active charcoal, suggesting that still other radical scavenging compounds are present in the crude extracts. Active charcoal also removes the steviol glycosides from crude extracts [12]. However, the identity of the other compounds removed by the charcoal remains unknown (flavonoids, vitamin). A complete analysis of the compounds including an ADME study is required to relate pharmacologic effects to specific compounds of the mixture.

Table 1: IC50 values in mM of different radical scavengers.

Radical	•OH	Superoxide	TBA	DPPH	NO
Ascorbic acid	1.15	0.059	11.3	0.055	0.01
Quercetine	0.12	0.32	0.91	13.817	0.18
Stevioside	0.22	1.491	323	-	
Rebaudioside A	0.2	2.529	288	-	
Steviol glucuronide	0.21	0.211	149	-	
Hydroxytyrosol	1	0.51	2.34	0.186	34.3
Metformin	0.97	0.993	104	-	-
Aspirin	0.31	0.904	23.2	-	-

Conclusion and Working Hypothesis

To explain the myriad of beneficial effects of steviol glycosides and steviol glucuronide, a common trigger had to be found that was responsible for all the effects. Radicals and ROS scavenging activity of steviol glycosides and steviol glucuronide are the possible common trigger involved. Moreover, it is known that steviol glucuronide can be found in the peripheral blood at sufficient elevated concentrations to show radical scavenging in vivo (between 2 and 8 μg/ml plasma (± 5-20 μM) after a daily dose of 3 mg × 250 mg stevioside) [23,24]. It has strong ROS scavenging activity and it can be transported all over the body. By its ROS scavenging, it can positively influence the above cited diseases, as these are in some way related to excess of radicals. It is known that by their ability to decrease oxidative stress in tissues, antioxidants can improve or prevent diseases, e.g., the serum liver enzymes are improved by α-tocopherol (vitamin E) [42]. Due to its potent anti-inflammatory activity, γ-tocopherol was more effective than α-tocopherol in treating diseases involving oxidant stress and inflammation [43].

Crude *S. rebaudiana* leaf and stem extracts showed very potent radical scavenging activity towards both ROS and RNS [44]. This might explain why crude leaf extracts were more efficient in the care of type 2 diabetes as shown by [14;31-34].

Since thousands of years, natural agents have been used to treat many diseases. Recently, whole leaf extracts of Stevia were very effective against various morphological forms of *Borrelia Burgdorferi* in *in vitro* cultures [45]. Stevioside was inactive, but this was probably due to the lack of absorption due to the large size of the stevioside molecule [19]. Because of the lack of a targeted therapy and as crude Stevia extracts as well as steviol glucuronide have an excellent radical scavenging activity, and because of known effects on decreases of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, Stevia might be an excellent drug for fighting the sepsis in COVID-19 patients. Administering steviol glycosides at values corresponding to the ADI (e.g., daily 3 mg × 250 mg stevioside), a plasma concentration between 5 and 20 μM glucuronide could be found. This amount can certainly have some beneficial radical scavenging effects. Its concentration can be doubled by increasing the intake, without provoking health problems. Crude Stevia leaf water extracts might still be better to treat the sepsis as these extracts contain the group of steviol glycosides, as well as many scavenging compounds like vitamins, polyphenols, flavonoids and other unidentified compounds. Unfortunately, at this stage it might not be possible to identify the possible active compounds of the crude extracts. More work including ADME studies are required. Nevertheless, it might be worth trying these extracts already as no risks are to be expected. Studies have shown that up to 5g of dried Stevia leaves are well tolerated and have no harmful effects on human health [14]. As Stevia is not a proper drug, it can be used preventively to improve the general health due to its special properties and this specifically in the risk groups for COVID-19, namely patients with obesity, diabetes, hypertension [12-14].

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