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An Insight into attributes of *Stevia rebaudiana* Bertoni: Recent advances in extraction techniques, phytochemistry, food applications and health benefits

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ABSTRACT

Stevia rebaudiana is a sweetener herb belongs to Asteraceae family, native to Argentina, Brazil and Paraguay. Stevia has potential qualities of a sweetener and also constituting a source of many substances with a nutritional effect on the human beings. The leaves of stevia contain stevioside, rebaudioside, steviolbioside, and isosteviol and sweeter than sucrose with zero calories. These steviol glycosides considered for the sweet taste and have commercial value globally as a sugar substitute in foods, beverages and nutraceuticals. The present article provides an overview of different extraction methods, phytochemistry, commercial application of stevia in various products such as confectionary products, bakery, dairy and beverages. Various studies shows promising health benefits of stevia against diverse aliments such as anti-microbial, anti-obesity, anti-cancer, anti-oxidant, anti-hypertensive, anti-diabetic properties considered in the present paper. Clinical studies revealed that steviol glycosides, which are an essential phytochemicals of stevia is safe for human consumption with no acute and subacute toxicity. This study could provides a new direction of stevia for treatment of human diseases and contribute in innovative stevia-based products.

1. Introduction

Stevia rebaudiana Bertoni (family Asteraceae) is a natural plant cultivated in many regions of the world. Almost 200 species of stevia are known, but of all those *S. rebaudiana* is the only one possessing sweet taste [1]. Honey leaf, sweet leaf and candy leaf are few terms used synonymously with this plant. It almost tastes 200–300 times sweeter than sucrose [2]. It is a good source of vitamins, essential amino acids, minerals and fatty acids [2].

Stevia has gained its importance as a natural sweetener in many functional foods such as dairy, drinks, bakery, confectionery. Stevia have its own distinct taste and sweetness profile which is now frequently using in various commercial products [3]. As the mortality rates are increasing recently due to the prevalence of heart disorders, obesity, stroke and Type II diabetes. Thus, more attention has been shifted towards the nutrition and foods having low glycaemic index and that too with natural origin [4]. International stevia council have reported more than 100 countries acquired the approval for stevia and its demand is increasing day by day [5].

Regulatory modifications in EU has also led to proliferation in demand for stevia in Europe [6]. West countries holds the largest food and drink market globally and natural sweeteners such as stevia is the most desirable approach for Europe market [7]. As per the New Nutrition Business Survey 2020, approximately 66% of European consumers have attempted to decrease their sugar intake and look forward for sugarless products [8]. This highlights the demand for natural sweeteners which also comprise the functional characteristics that helps in health benefits and managing blood sugar levels and obesity [9].

Stevia is broadly used in food industries as a sweetening substitute and is the main ingredient for many functional foods. Its market is growing consistently [10]. Additionally, it also has excellent therapeutic properties such as anti-diabetic, anti-hypertensive, anti-obesity, anti-caries, anti-tumour, anti-oxidant properties [11,12]. More interest has been developed in this plant due to its biochemical properties and its physical behaviour. Not only as a food supplement. Stevia is used as a texturizing agent for edible preparations due to its physic chemical

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characteristics [13]. These properties make stevia more desirable candidate for food industry. Novel strategies are being adopted from time to time to improve the taste aspect of this medicinal plant [14].

2. Botanical description and phytochemistry

Stevia belongs to the Asteraceae family better known as the sunflower family and incorporates shrubs and herbs of about 200 species. The plant can attain a height of up to 1 m [10]. The root system of this plant is widespread and leaves are brittle, elliptical and small with spatulate shape and tip of lamina is blunt [15]. The upper region of the leaf is more granular whereas stem possesses wood-like characteristics but is weak from the bottom. Small branches appear on its rhizomes [16]. The flowers exhibit pentamerous arrangement and colour fades from white to purple down the throat. Stevia can be well grown in extensive conditions of soil provided with sufficient moisture and drainage [17]. It can be cultivated when grown as a perennial shrub mostly in subtropical regions including some parts of the United States [18]. Locally, this plant is found in northern parts of South America and also near the Highlands of Amambay. The major cultivator regions include Continental China, Malaysia, Korea, Brazil, Thailand, Taiwan, Israel, Philippines, Canada, UK, Ukraine. Stevia is an annual plant and is grown in regions favouring yield of leaf and stevoside content [19].

Seed fertility is based on the insect pollination and large-scale cultivation becomes challenging. Hence stem cuttings are more reliable methods for the propagation purpose. In addition to this tissue culture is considered best for the quick propagation [20]. The moisture demand for the stevia plant is extensive and it grows well in summers and late spring [10]. About 1000–1200 kg of leaves and 60–70 kg of the stevoside content can be obtained from 1 ha of the plantation. Although the yield is low but 70 kg of stevoside sweetening potential is equivalent to 300 times of that of saccharose and 21,000 kg of sugar yielded from 1 ha [21].

When it comes to the therapeutic value of the plant, it lies in its bioactive components. Stevia includes a definitive blend of polyphenols, terpenes, flavonoids, tannins and alkaloids [22]. Stevosides are the major diterpene glycosides found in *S. rebaudiana,*and is responsible for its characteristic sweet taste which adds to its botanical value as an alternate for artificial sweeteners with more potential and promising results [23,24].

3. Phytochemistry of stevia

The primary glycosides present in the stevia plant are rebaudiosides, dulcosides, steviosides, steviomonoside and steviolbioside, among them stevoisides are the most abundant. These are biosynthesized in leave tissues from kaurenoid precursor with gibberellic acid and structurally belong to tetracyclic diterpens. These steviol based diterpene glycosides are recognized as stevioside (4–13% w/w), rebaudioside A (2–4% w/w), B, C (1–2%), D, E and F, steviolbioside, dulcoside (0.4–0.7%). Stevia glycoside are the secondary metabolites whose structures are based on their aglycon core commonly known as steviol or *ent*-13-hydroxyur-16-en-19-oic acid (Fig. 1). These differ by type and number of sugar molecule associated with them as shown in Fig. 1 below [25–28].

4. Biosynthesis of stevia glycosides

The biosynthesis of stevia glycosides (SGs) takes place in the chloroplast in the plant's tissues. The biosynthesis of stevia glycoside is depicted below in Fig. 2. The biosynthesis of SGs is highly regulated by the development of chloroplast membrane i.e., only the plants having well developed leaves are able to synthesize stevia glycosides in desired quantities via their specialized cells and structures [27,29,30]. The leave of *S. rebaudiana* biosynthesize eight glycosides which are prominently derivatives of tetracyclic diterpene known as steviol. There are sixteen enzymes which are involved in the biosynthesis of SGs and the pathway follows the steps which are involve in the gibberellic acid (GA) methylerythritol-4-phosphate (MEP) pathway which is used for the synthesis of isoprenoid and kaurenoic acid [31–33].

The initial steps for the biosynthesis of SGs occurs in plasmid i.e., via multistep methylerythritol 4-phosphate (MEP) pathway, in which the primary metabolic products pyruvate and glyceraldehyde-3-phosphate are converted to a geranylgeranyl diphosphate via the formation of 1-deoxy-D-xylulose phosphate, 2-C-methyl-D-erithritol-4-phosphate (MEP) cytidine-5-phosphate (CTP), methylerithritol cytidine diphosphate (CDP-ME), 4-diphsophocytidyl 2-C-methyl-D-erithritol-2-diphosphate (CDP-ME), 2-C-methyl-D-erithritol-2,4-cyclodiphosphate (MEcPP), 4-hydroxy-3-methyl-butenyl-1-diphospahte (HMBPP), Isopentyl disphosphate (IPP) and Dimethylallyl disphosphate (DMAPP). The process involves the DXS, DXR,CDP-ME synthase, CDP-ME kinase, Cyclodiphosphate synthase, HMBPP synthase, HMBPP reductase, Isomerase and GGPP synthase. The formed geranylgeranyl diphosphate

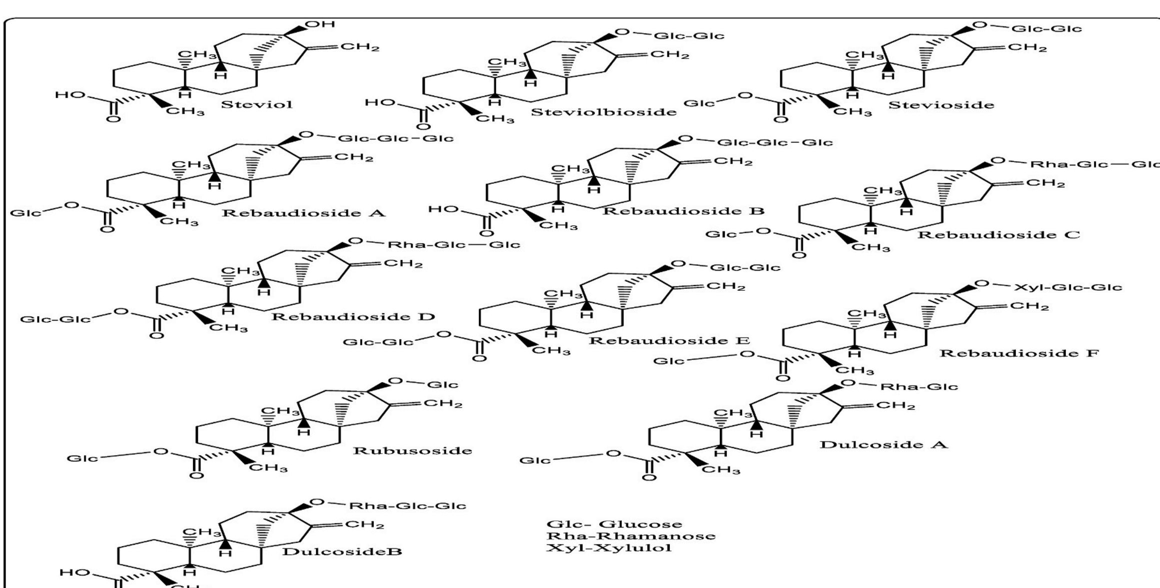

Fig. 1. Structures of different chemical compounds reported from Stevia.

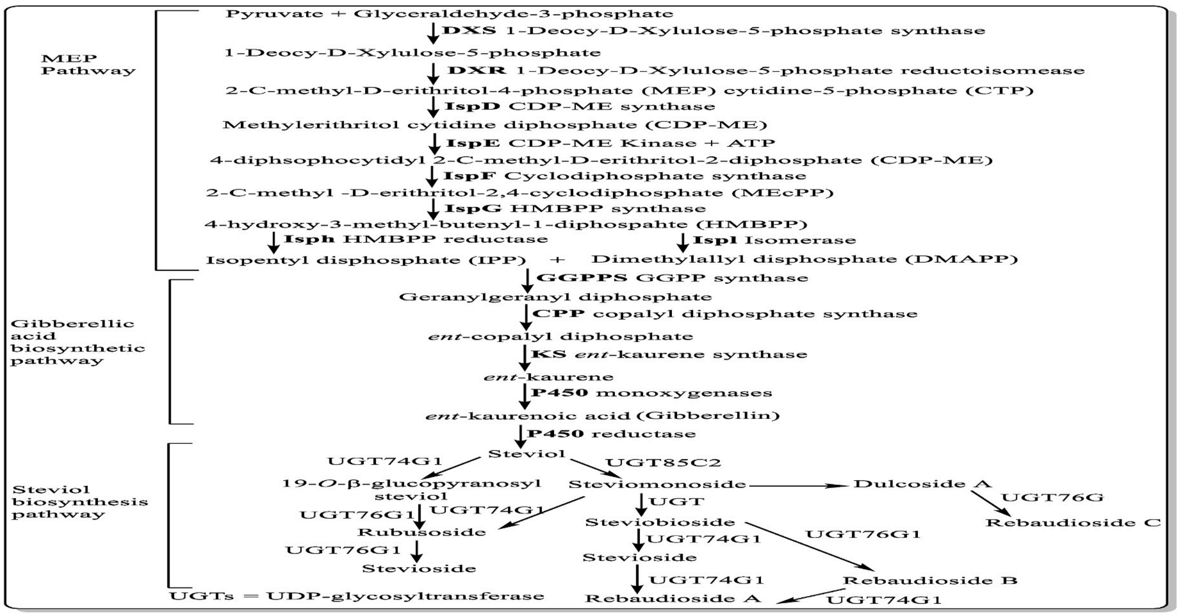

Fig. 2. Biosynthesis of stevia glycosides.

is then converted to *ent*-copalyl diphosphate via cyclization in the presence of enzyme CDP synthase, which is then underwent ionization cyclization in the presence of kaurene synthase (KS) to for *ent*-kaurene. The formed *ent*-kaurene is then transferred to endoplasmic reticulum wherein the oxidation by P450 monooxygenase, lead to formation of *ent*-kaurenoic acid or gibberellin which is converted to steviol by the action of P450 reductase. After the formation of steviol, a series of glycosylations, usually catalyzed cytosolic UDP-dependent glycosyltransferases (UGTs) takes place in the cytosol, leading to the production of different stevia glucosides (Sgs) [28,34–37].

5. Caloric content of steviosides

It has been observed that SGs are not bio transformed by human body via oral route and no gastrointestinal enzyme can help in degrading its glycone part to aglycone part (steviol) [38]. This similar phenomenon can also be seen with pigs and rabbits. However, *In vitro* studies demonstrated that bacteria present in human colon can biotransform the stevioside but this has not been demonstrated in *in vivo* studies. However, majority of the SGs are absorbed and conjugated via glucuronidation in the liver [39].

Various studies on digestion and absorption have been done with SGs and no effect of digestive enzymes and gastric juices has been seen on the digestion pattern [40]. In a study to monitor the digestion pattern of stevioside, it was observed that digestive enzymes does not contribute to the degradation of SGs which are hydrolysed by intestinal microbiota in steviol and steviol-16,17-α-epoxide which again is converted to steviol and is eliminated as steviol glucuronide in the urine [41]. This is the reason why *S. rebaudiana* does not increase the calories in human body. A comparative profile of stevia and other sweeteners are given in Table 2.

6. Extraction methods

Many methods have been developed for the isolation of SGs from the plants which majorly employs extraction with water or alcohol, followed by purification, partitioning procedures, phase extraction and column chromatography [42]. Specific protocols have been also developed to obtain steviol glycosides from physiological samples [43]. Maceration and thermal extraction are the most conventional methods used by manufacturers for the extraction of these glycosides. And in order to obtain products with better quality and yield, various intensification methods are used including supercritical fluids, ultrasonic baths as well as microwaves [44]. Most promising approach of all is multilayer membrane process developed with inbuilt function to concentrate sweetener principles from glycosides on a large scale with an additional benefit of excluding the bitter principles that deteriorate the taste of the product [45]. Extraction, purification and separation are the methods carried out sequentially to extract the Sgs on an industrial scale specifically from the leaves [46].

Although many variations occur at the different stages of the extraction but commercially 90% of yield of SGs is obtained along with 80% yield of the 2 principal glycosides, namely stevioside and rebaudioside A. Recrystallisation together with preparative RP-HPLCto get extremely pure compounds. Another method is amino column using which isolates glycosidic compounds post aqueous extraction as per their molecular weights to get better resolution. Chloroform-methane, propylene glycol and glycerol are the solvents employed for isolation of stevioside at pilot-scale [47,48]. Different methods for extraction are describe in Table 1 and enlisted in Fig. 3.

7. Processing and manufacturing

Taste still remains the major challenge as the major sweetening agent in stevia is rebaudioside A, which leaves a bitter after taste leaving sugar still the better preferred option [49]. However, conventional breeding programs, advanced farming methodologies, development of newer

Table 1
Differentiate between Stevia and other artificial sweetener.

Stevia	Artificial sweetener
Zero calories	Rich in calories
Zero glycemic index	High glycemic index
Heat stable upto 160 °C	Less heat stable
Easy to digest	Not properly digested
Not metabolised and excreted through normal elimination channels	Metabolised and absorbed in blood
High safety	Low safety
Good stability	Low stability
Good solubility	Low solubility
Non-carcinogenic	Carcinogenic
Anti-oxidant properties	Not have anti-oxidant properties

Table 2
Different extraction method along with solvents and their yield value.

S. No.	Steviol Glycosides	Extraction methods/Condition	Solvents	Yield	Reference
1.	Stevioside	Hot extraction (material leaves) Drug solvent ratio: (1:15–1:75)	hot water (65 °C)	7.53%	[13]
		Hot Extraction (material leaves) Drug solvent ratio: (1:15–1:75)4:1	methanol	94.90%	
		Hot Extraction (material leaves) Drug solvent ratio:4:1	Methanol and water (4:1)	92.34%	
2.	Reb A	Extraction: temperature (30 °C) and sonic power (300–480 W) Solvent: isopropyl alcohol concentration (60%, v/v), time (6–24 min)	Isopropyl alcohol	35.61 g per 100 g leaves	[113]
3.	Stevioside and Reb A	Leaf material was refluxed with 2L methanol for 1 h. The extracted residue was dissolved in methanol.	Methanol	57.50 g	[47]
4.	Stevioside	Extraction: enzyme-assisted extraction Three different enzymes: pectinase, cellulase and hemicellulase Five different concentrations (0.5%, 1%, 2%, 3% and 4%, w/v) Extraction temperatures: (35, 45 and 55 °C for pectinase; 40, 50 and 60 °C for cellulase; 50, 60 and 70 °C for hemicellulase) Time: (15, 30 and 45 min).	Enzymes (pectinase, cellulase and hemicellulose), at five different concentrations (0.5%, 1%, 2%, 3% and 4%, w/v)	highest recovery of stevioside was achieved in 1 h at 60 °C, using hemicellulase	[114]
5.	Stevioside and Reb A	Cold extraction: Time: 12 h	methanol, ethanol and water	6.54% of stevioside and 1.20% of Reb-A	[115]
		Ultrasound extraction was carried out at Temperature: 35 ± 5 °C Time:30 min	methanol, ethanol and water	4.20% of stevioside and 1.98% of Reb-A	
		Microwave-assisted extraction (MAE) Power level: 80 W for 1min at 50 °C	methanol, ethanol and water	8.64% of stevioside and 2.34% of Reb-A	
6.	Reb D	Purifying Rebaudioside D from the Stevia rebaudiana Bertoni. Solvent: alcohol-water solution.	alcohol-water solution	8.8 g and it contains 98.4% Reb D	[116]
7.	Reb M	Hot extraction Temperature: 40 °C Time: 2 h	Water	1.1 g of Reb M with >98% purity by HPLC	[117]
8.	Steviol glycosides	Hot extraction Temperature: Higher For prime juice autoclaving was used.	Water	650 ml of dark brown primer juice.	[118]
9.	Stevioside and Reb A	Hot Extraction Method: rotary evaporator under reduced pressure, and finally lyophilized.	Water	Stevioside (16.4%) and Reb A (12.1%)	[119]
10.	Reb A	Ultra-sonication extraction technique ultra-sonication: Power 360 W Time: 12 min Solvent: Water	water	32.79 (g/100g)	[120]
		Ultra-sonication extraction technique ultra-sonication: power 360 W Time: 12 min Solvent: Ethanol	ethanol	33.85 (g/100g)	
		Ultra-sonication extraction technique ultra-sonication: power 360 W Time: 12 min Solvent: Isopropyl alcohol	isopropyl alcohol	37.10 (g/100g)	

extraction technologies have led in production of better-tasting stevia with more preferred taste profile and having a better market value [50]. So, this becomes a better market candidate keeping in the view the beneficial caloric values as well. The conventional method is to simply dry leaves and apply them in beverages such as teas and also in medicines for sweetening purposes with almost no side effects [51]. But these methods are no longer in use as this leads to green coloration of the preparation win unacceptable aroma due to the use of dry leaves. However, aqueous or water-based extracts which are further cooled and filtered are better preferred options. Other than extracts, crystalline powders are also used. There are numerous processes for refining patented in various countries which includes dissolution of sweetener in boiling water or any preferred solvent, followed by ion-exchange separation, filtration by coagulation of precipitation and crystallisation and drying. Methanol was the first solvent employed for the extraction and purification processes to improve efficiency and carry out better separation of stevoside. But use of methanol although removed in later stages has some concerns and hence under this process stevia is not fully categorised as a natural food product. Newer industries in other countries use only water-based extraction procedures with a purity claim of 95%, 96%, 97% and 98% and are considered as stevia crystals. And in order to increase the sales, crystals are often diluted with bulking agents such asmaltodextrin, lactose and even sugar sometimes to enrich the sweetening index. Typical administrative standards followed for stevia by India and other countries are summarized in Table 3.

8. *S. rebaudiana*: A functional component for food industry

As discussed, the therapeutic value of rebaudiana and sweetening potential, it is an attractive way for sweetening ingredients which can find its application in pharmaceutical industry as well for instance, its use as a solubilising agent. Its low caloric content adds to its value due to

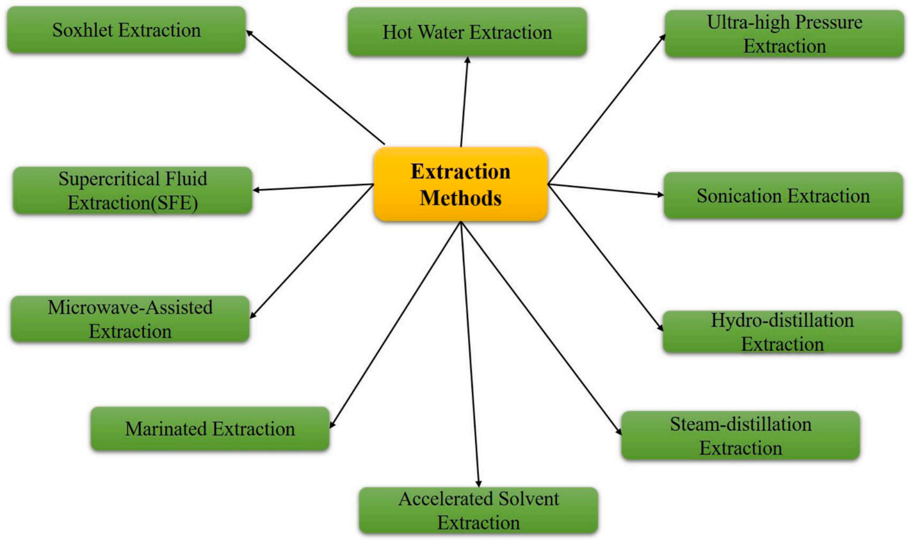

Fig. 3. Different extraction methods are considered for yield of Stevia.

Table 3
Average daily consumption in different countries as per regulatory authorities.

S. No	Country	Samples	ADI(mg/kg BW per day)	Regulatory Authority
1	Canada	Dietary exposure	50	Food Standards
		Fruits and vegetables juices	1100	
		Tabletop sweetener	40000	
2	China	Foodstuffs	170–10000	
3	India	Foodstuffs	200–3500	FSSAI
4	South Africa	Foodstuffs	330	EFSA

presence of diterpenoid steviol glycosides. Many countries have used it as a low-calorie sweetener for decades. And currently, Japan and Korea hold its largest market. Still 80% of stevia is grown in China. India to some extent also cultivates it and processes it as a sweetener.

8.1. *S. rebaudiana: As a food additive*

Due to its pleasant, refreshing and mild liquorice like taste, stevia finds its application in medicinal as well as industrial areas. It is used in edibles to enhance flavour as well as their aroma. Leaves of stevia are employed in the preparation of sauces, herbal teas, coffee, fruits and salads. Sometimes its leaves are used to impart colour to the food preparations [52]. Dairy products such as yoghurts, ice creams, flavoured milk also contain its extract. Presence of bioactive components such as chlorophyll, tannins, polyphenols, carotenoids delivers more importance to the stevia for its applications in production of functional food as well as nutraceuticals [53].

Insulin: a fructan type polysaccharides extracted from roots of stevia as demonstrated its applications as prebiotic nutrient in production of functional food [54].

8.2. *As a non-caloric sweetener*

Due to growing health concerns, food industry is in demand to replace sucrose and artificial sweeteners from the food and beverage preparations but without having sheer impact on the taste perception [55]. Replacement is even more embraced if the sweetener is non-nutritive and natural in source [56]. This replacement can be highlighted in the following sections.

8.3. *As a functional confectionary product*

Replacement of sucrose with stevia in the bakery products such as muffins, cakes, biscuits, cookies have significantly decreased the glycaemic index in *in vitro* studies and hence improved its nutritional value [57]. But certain authors have reported the unacceptable after taste in the stevia substituted products [56]. So, a study was conducted where stevia was added along with 50% of sucrose in addition to vanilla and cocoa powder as flavouring agent and it was compared with those products having 100% of stevia. It was found that the 50% replacement has preserved the texture and sensory characteristics as per the sensory acceptance test. Similarly, oatmeal cookies were prepared containing stevia extracts in 25%, 50%, 75% and in 100% of sucrose. And as per the sensory acceptance test 25% and 50% sucrose added cookies have the favourable sensory and texture characteristics likewise [58], sweet bread has also been tested in the same way containing 50% sucrose and SGs and 100% of SGs respectively and putting together all the aspects 50% sucrose and SGs combination has found more relevance and acceptance in terms of physical and sensory characters [59].

On the other hand, functional whipping creams are developed using isomalt with rebausioside A in place of sucrose to be used in the food products such as pastries, cakes, desserts and coffee. This is a cost-effective option to develop a low caloric sweetener for safe and healthy use [60]. Milk and milk products are most abundantly used in the dairy productions and fluid extensive use in day to day life but also contain sucrose in large quantities so flavoured yoghurts have been developed with the auxiliary use of stevia in combination with sucrose to balance the preceptory profile along with the health benefits [7]. Similarly, desserts such as ice creams have been developed in the similar way by maintaining the proportion of sweetener as well as texture [61]. So, in this way stevia has find its extensive application in dairy as well as bakery products revolutionising the old belief of sweetener products to be perceived as deleterious to health.

8.4. *As a functional substitute for beverages*

A large amount of sucrose is used in many beverages. Stevia is a potential agent for sucrose replacement in various beverages. Stevia has been used as a beverage in drinks like apple cherry fruit juice, peach juice and many other fruit juices [62]. In a study, authors recorded 25% reduction in calories when −44% of sucrose was replaced with 160 mg/L of steviosides in a peach juice, without modification of taste [63]. In a more recent, investigation, peach juice has also been prepared by completely replacing sucrose with 20 mg/100 mL stevia and also maintains its physical characteristics along with sensory acceptance [64]. But on the contrary passion fruit juice sweetened with stevia has bad after taste and overall lower acceptance [65]. however, for such cases additional flavours such as lime flavours are suggested [66]. Similarly, in another study mango nectar has been produced by replacing sucrose with 3% w/w stevia and 6% inulin as texturizing agent which nectar with all the desired physicochemical and organoleptic characters [67]. Another very recent study has produced chokeberry juice with the incorporation of stevia leaf powder and produced a drink with a good blend of vitamin C, chlorophyll, polyphenols and carotenoids maintaining the nutritional value of the beverage [68]. Even the most popular brands of carbonated beverages like Pepsi, Coca Cola Co., and Atlanta have introduced stevia-based beverages [69] where Coca Cola Co. and Pepsi introduced stevia sweetened low caloric beverages named Stubborn Soda and Cane Sugar respectively. Furthermore, iced tea containing stevia has also been produced and approximately 300 teas have been produced since 2010 containing stevia which are formulated as low-calorie teas [70].

8.5. Dairy: Stevia is used as an alternative for sucrose

Recently, considerable increase of demand for stevia based dairy products due to its antioxidant property and found against various diseases. Besides, stevia reduces the appetite and may support to diabetic people for the regulation of blood sugar. The addition of stevia based ingredients in dairy products may reduces the sugar amount and can able to control cholesterol. Nowadays, population is suffering from obesity, weight gain, and other metabolic disorders, stevia can be one of the choice in dairy based products. Stevia may consider as a rising star in the sugar space, as it provides dairy manufacturers a label-friendly and zero calorie option [71]. The dairy industry has strongly emphasized the use of stevia in order to decrease childhood obesity and sales of nutritious supplemented milk, the NMPF- National Milk Producers Federation and IDEA- International Dairy Association has petitioned FDA to modify definitions of milk and other dairy products. This will revolutionise the dairy industry.

8.6. As a polymer for edible and soluble packings

The increasing plastic pollution due to disposal of food packings is a growing concern and hence it becomes extremely essential to address the problem. So, the polymers which are biodegradable are developed from time to time but production of such bioplastics need special industrial treatment [72]. Stevia due to its anti-microbial and nutritive properties have been gaining relevance in production of sweetened edible films for packaging of drinks. So, recently a study has been done on stevia rebaudiana where its addition has been used as biofilm along with alginate the material thus obtained imparted five homogeneities, good flexibility elasticity and strength. Even when stevia was added in less quantities, it imparted reproducible and environment friendly effects because of its renewable nature [73].

9. Tabletop sweeteners

The innovation, product improvement, quality and customer satisfaction are the prime objectives of food and nutraceutical industry. The demand of sugar consumption is continuously increasing globally. Therefore, to serve consumers who are always search sweet taste, tabletop sweeteners have appeared in the market. These tabletop sweeteners marketed by food companies and well appreciated by the consumers. These Tabletop sweeteners may contain the bulking agents depends to the company's formulations such as starches, modified starches, dextrose, fructo-oligosaccharides, isomalto-oligosaccharides, fructose glycerol isomalte, lactose, maltitol, maltodextrin, mannitol, polyethylene glycol, propylene glycol, sucrose, sorbitol; and others [74]. Tabletop are the low-caloric choices to be used to hot or cold beverages or for sprinkling purposes and for other purpose as well as recipes suitable for patient of various disorders. Major factors to be considered include sweetness, stability, texture in addition to colour, taste, aroma and consistency. Stevia incorporates all these characteristics leading to be the manufacturer's demand.

10. Innovation provides opportunities

There have been many innovations in the food and beverage launches involving stevia which has increased by 26% in 2018 in European market. New launches in the innovative products provides better opportunities for the stevia exporters in the developing countries. There is an upsurge in then demand of more natural and healthier stevia-based products in foods supplemented with stevia as well as beverages. Thus, innovation is very much necessary to fulfil the consumer demands in order to provide alternatives for the natural sugar. Many well-known companies have launched the stevia-based products including the Danone group, PepsiCo, Coca-Cola Co, Nestle, Ricola and Unilever. Reb M stevia is a sweetener launched as TASTEVA® utilising stevia by Tate & Lyle which is a leading European sugar company. Hence, innovative approaches are a milestone for stevia industry.

11. *S. rebaudiana*: A therapeutic sweetener

The market value has even doubled due to the fact that stevia even includes a very good therapeutic index in the era of rising prevalence of diseases such as diabetes, obesity leading to more stevia related product launches. Fig. 4 gives an account of the major pharmacological properties of the stevia.

11.1. Diabetes

It is presumed that diabetes is implicated by pancreatic beta cell dysfunction and insulin resistance. Various preclinical studies support the scientific ability of stevia to enhance the insulin sensitivity and improve impaired glucose metabolism. The animal studies support that chemical constituents of stevia has a significant role against diabetes [75]. Further, the mechanism describe that stevia reduce the plasma glucose concentration and delaying the development of insulin resistance [76]. Various studies revealed that stevia have the ability to promote insulin secretion and reducing islet inflammation [77]. The molecular mechanism of anti-hyperglycaemia illustrate that due to insulinomimetic activity of stevia and its bioactives may effect the direct activation of glucose transporter molecules and improved glucose uptake and absorption [78]. Experimental studies also suggest that stveia regulate the protein expression for glucose uptake in peripheral tissues and play an important role in insulin-independent pathway. Additionally, clinical study also propose the anti-diabetic activity of stevia [79]. Some the most recent clinical trials as follow:

(I) -Sambra V et al. (2021), examine the effects of D-tagatose or stevia preloads on carbohydrate metabolism markers after an oral glucose load, as well as subjective and objective appetite in women with insulin resistance (IR). Randomized controlled crossover study. Women with IR without T2DM ($n = 33$; aged 23.4 ± 3.8; BMI 28.1 ± 3.4 kg $\times$ m^2) underwent three oral glucose loads (3 h each) on three different days. Ten min before oral glucose load, volunteers consumed a preload of 60 mL water (control), 60 mL water with stevia (15.3 mg), or D-tagatose (5000 mg). Serum glucose and C-peptide were evaluated at −10, 30-, 60-, 90-, 120-, and 180-min. Subjective appetite was determined with a visual analog scale. Food intake was measured at ad libitum buffet after 180 min. C-peptide iAUC was significantly higher for stevia (median (IQR): 1033 (711–1293) ng $\times$ min $\times$ L^{-1}) *vs.* D-tagatose (794 (366–1134) ng $\times$ min $\times$ L^{-1}; $P = 0.001$)

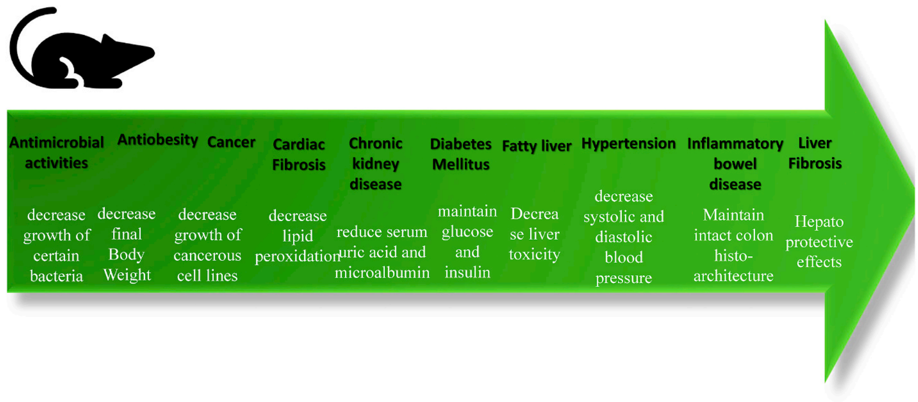

Fig. 4. Major pharmacological properties of the stevia.

or control (730 (516–1078) ng × min × L^{-1}; P = 0.012). At 30- and 60-min serum glucose was higher for stevia vs other conditions (P < 0.01). Volunteers reported greater satiety for stevia and d-tagatose vs. control at 60 min and greater desire to eat for stevia vs. control at 120- min (all P < 0.05). Objective appetite did not vary by condition (P = 0.06). Findings suggest that these NNS are not inert. Stevia intake produced an acute response on C-peptide release while increased serum glucose at earlier times. It is possible that NNS affects subjective but not objective appetite [111].

(ii) -Stamataki NS et al. (2020) examined this randomised, controlled, open-label 2-parallel arm trial examined the effects of daily stevia consumption on glycaemia in healthy adults. Secondary endpoints included body weight (BW) and energy intake (EI). Healthy participants (n = 28; aged 25 ± 5y, body mass index 21.2 ± 1.7 kg/m^2) were randomised into either the stevia group (n = 14)-required to consume a stevia extract daily-or to the control group (n = 14). At weeks 0 and 12, the glucose and insulin responses to an oral glucose tolerance test were measured; BW and EI were assessed at weeks 0, 6, and 12. There was no significant difference in the glucose or insulin responses. There was a significant main effect of group on BW change (F (1,26) = 5.56, p = 0.026), as the stevia group maintained their weight as opposed to the control group (mean weight change at week 12: 0.22 kg, 95% CI [-0.96, 0.51] stevia group, +0.89 kg, 95% CI [0.16, 1.63] control group). The energy intake was significantly decreased between week 0 and 12 in the stevia group (p = 0.003), however no change was found in the control group (p = 0.973). Although not placebo-controlled, these results suggest that daily stevia consumption does not affect glycaemia in healthy individuals, but could aid in weight maintenance and the moderation of EI [112].

(iii) -Farhat G et al. (2019) investigated the effect of stevia on post-prandial glucose levels, appetite and food intake. Thirty participants (20 females/10 males; 26.1 (10.56) years; body mass index (BMI) 23.44 (3.42) Kg/m^2) took part in a three-arm crossover trial where they received preloads of water, sugar (60 g) and stevia (1 g) on three different days, followed by an ad libitum pizza lunch. Breakfast was standardised. A one-day diet diary was collected on each test day. Visual analogue scales (VAS) were used to assess subjective feelings of appetite. Blood glucose samples were collected at 30-min intervals until 120 min post lunch.Energy intake did not significantly differ between preloads for ad libitum meals (p = 0.78) and overall day (p = 0.33). VAS scores for hunger and desire to eat (DTE) were lower following stevia preload compared to water (p < 0.05). After adjusting for the sugar preload and calorie content, postprandial glucose levels did not significantly differ between interventions.Stevia lowers appetite sensation and does not further increase food intake and postprandial glucose levels. It could be a useful strategy in obesity and diabetes prevention and management [113].

Administration of SGs in diabetic rat models elicited dose dependent reduction in the blood glucose levels (0.5 mg/kg) and this was elucidated by augmenting the dose dependent pattern of insulin secretion and promoting the utilisation of glucose. In stevioside administered groups, the expression of a rate limiting enzyme in gluconeogenesis i.e., PEPCK-Phosphoenol pyruvate carboxykinase is reduced in a dose-dependent manner [80]. α-amylase and α-glucosidase are the regulatory enzymes for the digestion of carbohydrates and is important factor in determining blood sugar. In a recent *in vitro* assay done stevia leaves extract were found to be effective in inhibiting the α-glucosidase and α-amylase activity [81]. Recently, a novel glycoside having phenyl-ethanoid moiety called steviophethanoside along with four other phenylethanolyl glycosides have been discovered from stevia leaves which has shown stimulatory activity in INS-1 islet β-cells and thus may impart hypoglycaemic effects but more investigations are needed to elucidate its mechanism of action [82]. More recent studies demonstrate that steviol glucuronide is an essential metabolite for glucose and dose dependent stimulation of insulin and glucose levels [24]. All in all, based on *in vivo* and clinical investigations, stevia is a promising approach for diabetes associated pathology but still a deeper underlying mechanism is still needed to be elucidated.

11.2. Hypertension

Hypertension cannot itself be designated as disease but it is a major risk factor for the development of various life-threatening diseases including myocardial infarction, congestive heart failure, left ventricular hypertrophy, aneurysms peripheral vascular disease, atrial fibrillation, hypertensive neuropathy, retinopathy and stroke. Hypertension can remain asymptomatic for a long time, which is why it is sometimes referred to as 'silent killer'. Antihypertensive medications and modifiable lifestyle changes can reduce hypertension and related complications if detected early [83,84]. Stevia as per various studies is used as a heart tonic to regulate heartbeats and normalise troubled blood pressure. In a clinical study about 106 hypertensive women were given 0.25 g of SGs thrice a day and experienced both systolic as well as diastolic blood pressure keeping the lipid and glucose levels normal. Similarly, SGs have demonstrated significant reduction in systolic blood pressure but not much effect was seen on the diastolic blood pressure [38]. The hypothetised mechanism includes the blockage of calcium ion influx inside the smooth vascular muscle cells [85]. Another proposed mechanism is the inhibition of angiotensin converting enzyme as protein hydrosylates obtained from stevia leaf strongly inhibits the activity of angiotensin converting enzyme [86].

11.3. Obesity

Obesity is a major health factor and the starting point of many health-related complications and has multifactorial aetiology. This could be genetic or due to poor lifestyle and eating habits. High intake of sugar-based foods also contributes to its progression and thus it becomes reasonable that substitution of high caloric food and beverages is important in maintaining weight. Stevia fits in the role of non-caloric sweetener which is better than calorie loaded sweeteners such as sucrose. However, stevia imparts sweetness which is almost 100–300 times more than that of sucrose [5]. Even in practice a study has found reduction of total cholesterol, low density lipoproteins, triglycerides and body weight and increase in high density lipoproteins in rats given stevia and the opposite trend is found in those exposed to sucrose [87]. In a more recent investigation rats given the aqueous extract of stevia leaves well demonstrated to have better caloric profile ultimately reducing their overall body weight due to decreased intake of feed [88]. But yet promising results have not been seen in human subjects. Additionally, stevia preload is found to reduce the desire to eat and self-reported hunger in healthy volunteers [89]. Mostly, foods and beverages are supplemented with artificial sweeteners which are non-caloric but they are deleterious to health as they cause weight gain, brain and bladder cancers and many other complications [90]. Thus, it is necessary to argue the preference of natural sweeteners such as stevia for its safe and healthy use.

11.4. Antimicrobial agent

Due to high raising distress of re-emerging infection resistance developed in response to the antibiotics, there is a serious need to develop and discover new agents possessing antimicrobial properties [91].

As raising interest in plant based drugs, phytochemicals obtained from natural sources are being screened for their therapeutic values and great importance is given to those possessing anti-microbial activities [92]. Similarly, leaves of *S. rebaudiana* have demonstrated activity against bacteria such as *Streptococcus mutans, Escherichia coli, Staphylococcus aureus, Bacillus subtilis.* This was specific for the methanol and chloroform extract. In addition, chloroform extract was also found to have pronounced anti-fungal effect and investigations reveals, it inhibits *Sclerotonia minor* fungus. Conversely, methanol extract of leaves is active against *S. minor* as well as *Curvularia* spp [93]. Likewise, aqueous extract of stevia leavesdoes possess anti-fungal activity but lacks anti-bacterial effect. Specifically, chloroform, ethyl acetate and acetone based extracts were evaluated and found to be active against microbes such as *Candida albicans, Aeromonas hydrophila, Vibrio cholerae, Salmonella typhi, Trichophyton mentagrophytes, Cryptococcus neoformans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Epidermophyton* spp [93]. This property of stevia inhibiting a spectrum of bacteria makes it a desirable candidate for treatment of cold, wounds, flu, gum issues, sores of traditional importance. And it is also beneficial for those suffering from yeast infections and recovering streptococcal infections [93]. These plants have inherent property to synthesize aromatic principles such as phenols and their oxygen substituted derivatives. This provides protection to the plants from microbial infections and prevents their death [92]. An elaborated application of extracts of stevia due to its anti-oxidant and anti-microbial property is its use as a preservative agent in various seafood-based products like salmon paste [94].

11.5. Dental caries

Dental caries is an infectious condition which progresses slowly which may be due to dwelling microbiota in oral cavity, intake of food products like carbohydrates, fermented products and variable salivary flow rate [95]. So, it is important to find a healthy substitute that can help in preventing caries. In a study, 10% stevia extract after being rinsed on dental plaques has shown reduction in the plaque when compared to 10% sucrose solution rinse. In addition to this stevioside and rebaudioside A has shown low formation of *S.mutans* biofilm after rinsing and also found to be non-acidogenic in nature [96]. The non-fermentable nature of stevia solution hinders the bacterial survival [97]. Altogether, stevia has shown long term safety in dental caries.

12. Anti-oxidant agent

Molecular species having unpaired electrons are termed as free radicals and are very reactive and unstable. In low to moderate concentrations, they do not have much effect, but at higher concentration they creates a state of oxidative stress [98] which is very detrimental for human health and promotes development of diseases such as neurodegenerative disorders, carcinogenesis, cardiovascular disorders, inflammatory bowel diseases, obesity, osteoporosis [99]. For this cause anti-oxidants have gained its significant role due to their property of preventing damage caused by oxidative stress. They interrupt the oxidative process by neutralising free radicals, chelation with catalytic metals and by its scavenging properties [100].

Due to least toxic effects of edible plants, they are extensively being explored for the presence of anti-oxidant compounds [101]. Several investigations done on stevia extracts confirm that, leaf and callus extracts possess dose-dependent anti-oxidant potential [102]. Many studies reported the natural anti-oxidant potential of stevia extract in food industry as the quantity of polyphenols and flavonoids present in the ethanolic extract demonstrated neutralising property against $DPPH^{\bullet}$(1,1-diphenyl-2-picrylhydrazyl) and $ABTS^{\bullet+}$(2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radicals. And even higher amounts of these principles are present in glycol aqueous extracts followed by extract [103]. In a pre-clinical study, it was found that stevia residue extract obtained as a by-product from the production of steviol glycosides has shown protective effect against oxidative stress induced in aged mice via D-galactose by promoting the function of enzymes such as catalase, superoxide dismutase, glutathione peroxidises. It also happens to promotes the total anti-oxidant capacity and decreases acetyl-cholinesterase activity as well as levels of malondialdehyde in liver, blood and brain. This is elucidated to take place via activation of Akt/Nrf2/HO-1 pathway and thus gives a well desired candidate as a dietary supplement for alternating the oxidative stress which can be age-dependent also [104]. In another animal model Wistar rats, stevia leaf and powdered extract (4.0%) demonstrated reduced lipid peroxidation and favourable modifications in anti-oxidation markers in diabetic rodents [1]. Even production of the wheat bread containing extracts of stevia preserves its anti-oxidant potential and thus it falls in the category of functional food [12]. Similarly, exotic fruit beverages containing 1.25% and 2.5% (w/v) of stevia as a sweetener are also functional beverages as their anti-oxidant property is demonstrated to be better than beverages not containing stevia [105]. Likewise, the green stevia powder added to the strawberry-based juices have better total phenolic and flavonoid content and have superior anti-oxidant capacity in contrast to the juices without stevia. Moreover, sonication processing technique in stevia based strawberry juices highlights the importance of preservation simultaneously enhancing anti-oxidation capacity and sweetness of beverages [68]. Whereas, functional yoghurts containing 0.25%–0.5% of freeze-dried extract of stevia also possessed better total phenolic content and enhanced anti-oxidant potential in contrast to the ordinary yoghurt which helps in maintaining gastro-intestinal health [106].

12.1. Cancer

Cancer as per WHO 2018 is the second most leading cause of death and caused approximately 9.6 million deaths alone in 2018 worldwide. Development of new chemotherapeutics originating from natural sources is most desired. And steviosides obtained from stevia are natural based phytochemicals which has shown some promise as an anti-tumor agent and developing resistance in tumour cells [107]. The very first study was done on female rats with adenomas in mammary gland were given stevioside and were found to be shown anti-tumor activity. This has been seen in *in vitro* studies as well where the diterpene glycosides present in the ethanolic extract of stevia has shown anti-tumor effect in 3 cancer cell lines-colonic (HCT116), pancreatic (MiaPaCa-2) and cervix (HeLa) cancer cells. In addition to this CDK4, an enzyme produced by mutated gene related with development of cancers was inhibited by the extract, demonstrating its anti-tumor effect with the help of inhibition of CDK enzymes [54]. On the other hand, essential oil obtained from stevia has also shown cytotoxicity against rat glioma cells as well as Chinese hamster ovary cells which is comparable to vinblastine [108]. Similar effects were seen in gastrointestinal cancer cells [109]. Positive results were obtained in breast cancer cells as well (MCF-7 cells), and the proposed mechanism revealed that effect is due to apoptosis induction in MCF-7 and stevia and its bioactives could be is a desirable candidate to be studied for treating breast cancer [90]. Stevia is ability of inhibiting or reduce the growth of cancerous cell by inducing apoptosis and restraining cell proliferation on MDA-MB-231 and SKBR3 [110]. Another possible mechanism for this effect is thought to be inactivation of PI3K/AKT pathway along with the induction of apoptosis and cell cycle arrest which is elucidated due to anti-proliferative effects seen in ovarian cancer cell with the help of steviosides [107]. Altogether, these studies demonstrate the positive effect of steviol based compounds but more detailed clinical and pre-clinical trials are required to confirm its anti-tumour potential.

12.2. Renal function

Kidneys perform essential functions of body including homeostatic balance of body, maintenance of fluids and electrolytes. The steviosides derived from leaves *S. Rebaudiana* upon administration in hypertensive rats has shown to improve the renal plasma flow as well as glomerular filtration rate. It directly inhibits transepithelial transport of PAH-p-aminohippurate [111]. Steviol derivatives have shown inhibitory effect on cyst model of Madin-Darby canine kidney (MDCK) cells and this happens due to inhibition of CFTR-cystic fibrosis transmembrane conductance regulator. Hence, steviol analogues can be good candidates for treating polycystic kidney disease [112].

13. Conclusion

Recently, as the incidence of various metabolic disease has increased worldwide, there has been a great demand for new alternative low or no-calorie sweeteners across. In the market, different artificial sweeteners, but their use is limited due to reported harmful side effects. As a consequence, the search for natural sugar substitutes has led to the discovery of a number of substances that possess an intensely sweet taste or taste-modifying properties. *S. rebaudiana* is an agricultural crop cultivated and produced as a natural sweetener of high potency. It is a relevant raw material source of phytochemical constituents that promotes health and forms functional food ingredients. Diterpene glycosides play crucial role as sweetening agent and is currently in market as a source of natural sweetener in the beverages and foods. It is 200–300 times sweeter than other artificial sweeter currently used in market. Its non-toxic nature as per the clinical and pre-clinical data along with low caloric index justifies its use in food and beverage industry. There must be more emphasized on harvesting method, extraction techniques, yield value, purification and other commercial aspects. The newer variant of stevia based product could offer improved taste profiles, no or less side effects with improve blending techniques.

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Author contributions

Dr. Alok Sharma originated the idea and drafted the manuscript, Mr. Sitanshu, and Amrat Pal Singh substantially contributed in paper drafting. Dr. Alok Sharma and Dr. Marco Biagi contributed in the organized this manuscript and assisted in deriving figures and tables.

Declaration of competing interest

There is no conflict of interest.

Data availability

Data will be made available on request.

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