Review Article

Remedial Potentials of Sweet Leaf: A Review on *Stevia rebaudiana*

Dhanish Joseph[1*], Junia George[2]

1. Assistant Professor, Department of Pharmaceutics, Nirmala College of Pharmacy, Kerala, India.
2. Department of Pharmaceutics, Nirmala College of Pharmacy, Kerala, India.

***Corresponding author's E-mail:** dhanishjoseph707@gmail.com

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ABSTRACT

Stevia rebaudiana is a natural sweetest plant of Asteraceae family, also called sweet herb, sweet leaf, honey leaf, candy leaf. The steviol glycosides particularly stevioside, rebaudioside, steviolbioside are responsible for its sweet taste and hence can be used as a sugar substitute. Stevia has many therapeutic applications as demonstrated by various in vitro and in vivo studies like antibacterial, antidiabetic, antitumor. Besides as therapeutic agents, the constituents in stevia are lead molecules for further drug development.

Keywords: Asteraceae, anti-bacterial, hepatoprotective, sweet leaf, *Stevia rebaudiana.*

INTRODUCTION

The emergence of chemical molecules was a blessing at once to combat several diseases, but it paved the way for troublous situations like various adverse effects, the emergence of resistance. Researches on new molecules with the least toxic effects and better potency is on its way and more attention is being given upon traditional plants for forcing away the above problems. Thus in this work, a review on the medicinal properties on a perennial and endemic shrub, named Stevia rebaudiana is done.

Stevia rebaudiana is a shrub belongs to Asteraceae family and is indigenous to Paraguay, Brazil. It is being cultivated in some parts of Asia, Europe, and Canada. It is known for its sweetness imparted by its glycosides without causing any dysregulation. The phytochemical studies concluded the existence of tannins, alkaloids, glycosides, saponins, sterols, triterpenes with various potentials. [1, 2]

Antibacterial Activity

High -Performance Liquid Chromatography-Mass spectroscopy studies revealed the presence of antimicrobial compounds like flavonoids, terpenoids, dihydro deoxy streptomycin in the dried leaf extracts of Stevia rebaudiana. Antibacterial activity was found with all extracts prepared by different techniques like maceration, cold extraction, Soxhlet extraction. But enhanced solubility and extraction of compounds was found with microwave assisted extraction, as this method is superior over the other conventional techniques with respect to less extraction time, low volume solvent requirement, yield, time and energy consumption. [1,3,4 5]

Stevioside, which is a major secondary metabolite possess antimicrobial activity against various bacterial strains like *Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Escherichiacoli* and fungal strains like *Sclerotinia minor* and *Curvularia lunata*. Dilution of the crude extracts leads to impressive activity due to less viscous and better diffusion in the in-vitro culture medium. Absolute antibacterial activity is observed against *Staphylococcus epidermis* and ESBL (Extended Spectrum beta-lactamase) producing uropathogens which are resistant to conventional antibiotics. ESBL are plasmid mediated hydrolyzing enzymes that break the amide linkage of beta-lactam antibiotics like ampicillin thus contributing to resistance. But stevia synergized the activity of ampicillin and reduced its MBC (Minimum bactericidal concentration) from 10mg/ml to 200-400µg/ml. The mechanism of synergistic activity is not known so far and it needs to be confirmed by in vivo methods.[1,3,4]

Stevia rebaudiana can be used as a potent anticaries agent against Streptococcus mutans and Lactobacillus. Clinical studies revealed that 0.5%Stevia mouthwash is non-acidogenic, could lower the pH of saliva and improve the buffering capacity. Stevia reduced the microbial biomass which otherwise initiates biofilm formation as shown by 3-(4,5- dimethyl thiazol-2yl)2,5- diphenyl tetrazolium bromide assay. The anticaries activity of 0.5% Stevia mouthwash is significantly greater than 0.12% Chlorhexidine. [5,6]

Antiprotozoal Effect

Saponins exert antiprotozoal effect by its interaction with the sterol group present in the membrane of protozoa. The use of saponins against rumen protozoa is short-lived because saponins are deglycosylated to its aglycone called sapogenins by the rumen microorganisms, thereby it may become inactive. Stevia contains iminosugars which prevent the deglycosylation of saponins and increases their effectiveness. The major one is the glycoside inhibitor, 2,5- dihydroxymethyl-3,4-dihydroxy pyrrolidine(DMDP). [7]

Antioxidant Activity

Oxidative damage to biological materials is inflicted on biomolecules such as proteins, nucleic acid, lipids, and carbohydrates. Oxidative stress happens when there occurs an imbalance between the production of ROS (Reactive Oxygen Species) and the ability of our body to readily detoxify the reactive free radicals or to easily repair the resulting damage. In vitro, the antioxidant activity of Stevia leaves extract was confirmed by DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) radical scavenging assay, FRAP (ferric ion reducing activity) assay, and phosphomolybdenum assay. [8,9,10,11]

Both methanolic and aqueous extract of dried stevia leaves is enriched with polyphenols like hesperidin, ellagic acid, chlorogenic acid, eugenol, coumarin, vanillin and flavonoids and hence can be used as a potential source of antioxidant in food and beverages and a promising candidate for diseases like diabetes, cancer, neural disorders, arthritis and aging which is caused by the production of ROS(Reactive Oxygen Species). The potentiality of stevia antioxidants is able to supersede the synthetic antioxidants like BHA (Butylated hydroxyanisole) and BHT(Butylated hydroxytoluene), which recently limited in its use due to their carcinogenic potential. [8-14]

The antioxidant activity of phenolic compounds is due to the radical scavenging by hydrogen donation. Other radical quenching mechanisms include electron donation and singlet oxygen quenching. The antioxidant effects of flavonoids are ascribed to their power to neutralize the free radicals, chelate metal catalyst, activate antioxidant enzymes, reduce alpha-tocopherol radicals and prevent the actions of oxidases. A significant decrease in the cellular oxidation biomarkers like protein carbonyl content (PCC), antioxidant enzymes (SOD and CAT) was seen in the presence of stevia glycosides in CCl_4 induced oxidative stress in a fish model (Cyprinus carpio).[8,9]

Hydrogen peroxide, an abiotic stress elicitor resulted in an increased steviol glycoside production such as rebaudioside A and stevioside and nonenzymatic antioxidants that play a defensive role against an oxidative stress induced by hydrogen peroxide [15]

Wound Healing Property

Topical application of *Stevia rebaudiana* significantly enhance the wound contraction and re-epithelization rates in the short term as demonstrated in male Sprague-Dawley rats, in which incisions are made. Wound healing properties add stevia to the list of other similar groups of plants like *Ginkgo biloba, Morinda citrifolia.* It is suggested that the antioxidant nature of stevia contributing to the wound healing nature. The effects of stevia include:

- Acceleration of granulation tissue formation
- Increased number of fibroblast
- A more organized pattern of collagen fibers
- Greater tissue alignment
- Inhibited the activities of inflammatory cells and the production of chemical mediators [16]

Hemolytic Potential

Stevia rebaudiana decreased the hemolytic potential of *Listeria monocytogenes* which exhibits its pathogenicity by producing a pore-forming toxin Listeriolysin O, LLO which is responsible for lysis of primary vacuoles which then releases into the cytosol of host cells where it grows and multiplies leading to permanent host infection. [17]

Antihyperglycemic

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia accompanied by disturbances in carbohydrate, protein and fat metabolism, due to either insufficient insulin secretion or insulin insensitivity or both. Though *Stevia rebaudiana* is mainly used as a sweetening agent in foods and beverages, they do not induce a glycaemic response when ingested, rather than it exerts antidiabetic action by the enhanced secretion of insulin from the beta cells of pancreas and insulin sensitivity of peripheral tissues promoting glucose uptake. Stevioside also inhibits glucose production by inhibiting glucagon secretion as shown in alloxan induced rats. [18, 19]

The molecular mechanism of action of steviol glycosides are:

- Modulating pancreatic beta cell function by potentiating TRPM5, a Ca^{2+} activated cation channel, expressed on beta cells and peripheral enteroendocrine cells in the gut by accelerating insulin release in response to glucose stimulation. Steviol (aglycone part), stevioside, rebaudiosideA are not the direct agonist of TRPM5, but the steviol moiety is responsible for interacting with the protein. Besides, these glycosides can be used as antihyperglycaemic agents, they are novel leads to the development of antidiabetic drugs targeting TRPM5. Further, these agents won't produce hypoglycemia, as seen with synthetic agents and hence will be a great boon for diabetic patients.[20]
- Steviol glycosides are able to act as ligands of the insulin receptor(IR or IGF-IR) activating the P13k/Akt pathway. Upon activation, signal leads to the Glut 4 translocation from an intracellular pool to the plasma membrane, allowing glucose entry into cells and thus mimics the action of insulin. Biscuits incorporated with stevia was found to inhibit α- glucosidase activity. Aqueous extract of stevia produced antihyperglycemic and restore liver and muscle glycogen levels in hyperglycemia-induced rabbits by immobilization stress.[21,22,23]

- Comparison of stevia and pioglitazone, a thiazolidinedione, both of them having antioxidant properties too can act as ligands on PPAR-γ (peroxisome proliferator-activated receptor-γ), a nuclear hormone receptor and induce insulin secretion and control the level of blood glucose. The mRNA expression of PPAR-γ can be increased by both stevia and pioglitazone. The hypoglycemic effect is also aided by its antioxidant nature. [14]

- Reduction in the level of inflammatory cytokine IL-6, which potentiate the elevation of insulin resistance, and therefore helpful in type 2 diabetes. [24]

Stevia could control the neuronal synaptic plasticity in conditions of metabolic disorders induced by the high consumption of dietary fructose by influencing NOX-(NADPH oxidase level) specific targets and thus have a neuroprotective role. [25]

Antihyperlipidemic And Hypotensive Effect

Aqueous extract of Stevia rebaudiana exerts a hypolipidemic effect by

- Decreasing cholesterol and fatty acid synthesis
- Attenuating total cholesterol, triglycerides, and LDL levels
- Elevating HDL cholesterol [18, 23]

Stevia leaves help in regulating the blood pressure by relaxing arteries and prevent the buildup of calcium on artery walls, that promotes vasodilation and reduces total peripheral resistance and volume of extracellular fluid as result of elevated natriuresis and diuresis. Both hypolipidemic and hypotensive effect exerts a cardioprotective action. [26, 27]

Hepatoprotective

The antioxidant potential of Stevia can be utilized to alleviate hepatic injury like cirrhosis, hepatic carcinoma which is induced by the oxidative stress. The hepatoprotective ability of stevia is confirmed against CCl_4 induced and lipopolysaccharide-induced injury in rat and chicken embryo model. The mechanism of CCl_4 induced liver injury is its metabolic activation by CYP450 and forms trichloromethyl free radical $CCl_3^{\cdot}$. These free radicals stimulate lipid peroxidation, protein covalent binding. Glutathione depletion, and disturbance of calcium and iron ions ultimately leading to cell death. Lipopolysaccharide is an endotoxin, a potent inflammagen and the glycolipid component of the cell membrane of gram-negative bacteria. It exerts liver injury by releasing inflammatory cytokines like TNF-α (Tumor Necrosis Factor –α), IL-1β, IL-6 (Interleukins) & ROS. [28-30]

The molecular mechanism of hepatoprotective action of stevia is:

- Induction of Nrf2 pathway which is an endogenous pathway to reduce the level of reactive metabolites.
- Immunomodulatory action- by inhibiting NF-κβ that leads to the downregulation of proinflammatory cascade and thereby prevents necrosis, cholestasis, and preservation of liver parenchyma structure and function [28, 31]

Nephroprotective

Both stevioside and extracts of stevia show nephroprotective action due to the coinciding activities like suppression of oxidative stress, inflammation, and apoptosis. Renal hypertrophy, glomerular hyperfiltration are two known complications in the initial stages of DM as characterized by then increased cortical volume (80%)and its subcomponents PCT(Proximal Convoluted Tubule), DCT(Distal Convoluted Tubule), glomeruli, interstitial tissue rather than medullary volume. The molecular mechanism of these two complications are:

- Production of Transforming growth factor β (TGF-β) by mesangial components
- Overproduction of free radicals following hyperglycemia
- Expression of inducible nitric oxide synthase iNOS in response to cytokines [32,33,34].

Stevia and its glycosides attenuate not only diabetes-related kidney injury but also cisplatin-induced nephrotoxicity. Cisplatin is a chemotherapeutic agent which exerts its action by activating cell cycle arrest, apoptosis, and DNA repair.

The mechanism of nephroprotective action by:

- Attenuation of oxidative and nitrosative stress
- Antiinflammatory activity by decreasing p65 and TNF-α expression
- Antiapoptotic effect by suppressing the release of caspase-activating proteins
- Restoring cell cycle by reduced p21 expression and increased cyclin D1 expression
- Suppressing ERK1/2 activation, associated with apoptosis and cell cycle arrest. [33]

Antitumor Effect

Stevioside has shown a marked effect against various cancers like skin cancer, ovarian cancer and breast cancer as demonstrated in various cell line studies. The mechanisms for antitumor effects are:

- ROS mediated apoptosis
- Increased expression of apoptotic proteins like Bax, Bc1-2, caspase 9

- Reducing cell viability by inhibiting DNA synthesis and inducing cell apoptosis
- Isosteviol, a breakdown product of stevioside, manifested an inhibitory activity against the enzymes DNA polymerase and DNA topoisomerase II
- Inactivates P13K/AKT signaling pathway by inhibiting phosphorylation of P13 and AKT

From the methanolic extract of Stevia one compound was isolated and further confirmed by NMR to be centaureidin, which has an antimitotic effect to be used for tumor therapy.[11,35]

CONCLUSION

Stevia rebaudiana has become an important plant that needs to be commercialized without no time because of its therapeutic applications. Still, researches are needed to disclose its further potentials. The constituents of *Stevia rebaudiana* can be directly used as well as they permit the manipulation that drives to develop new synthetic versions or as drug leads.

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