

# The Research Progress on the Improvement and Safety of Steviol Glycosides in Dyslipidemia of Diabetic Rats

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**Abstract:** Steviol glycosides, as natural sweeteners, are highly favored in the market. Moreover, they have shown outstanding effects in improving dyslipidemia. Most diabetic patients experience dyslipidemia, significantly increasing their risk of cardiovascular diseases. Steviol glycosides can improve dyslipidemia in rats by enhancing lipid metabolism, improving insulin conditions, and exhibiting antioxidant and anti-inflammatory properties. There have been no reports of toxicity or allergic reactions from the use of steviol glycosides in the past, and extensive research currently confirms their safety. Therefore, steviol glycosides hold promise as an adjunct medicinal food for improving dyslipidemia in diabetic patients, promoting their application in the medical and food industries.

**Keywords:** Steviol glycosides; Diabetes; Dyslipidemia; Insulin; Safety.

## 1. Introduction to Steviol Glycosides

Steviol glycosides (SGs) are diterpene compounds found in *Stevia*, known for their high sweetness. As natural sweeteners, steviol glycosides are virtually calorie-free. The primary components are stevioside (STV) and rebaudioside A (Reb A). However, with the continuous pursuit of better-tasting sweeteners and advancements in extraction technology, other substances in steviol glycosides, such as rebaudioside M, have gradually replaced rebaudioside A to become the mainstream worldwide. Currently, steviol glycosides are widely used in the food industry across various countries, known as the "world's third natural sugar source" and the ideal "third-generation sugar source" of the 21st century [1]. Moreover, steviol glycosides have certain medicinal effects in clinical settings. Numerous experiments have demonstrated that steviol glycosides can lower blood pressure, reduce blood sugar, prevent dental caries, and exhibit anti-inflammatory and antioxidant properties. Steviol glycosides can also help obese individuals reduce cravings for sweets and fatty foods, aiding in weight control. Notably, in recent decades, steviol glycosides have shown remarkable abilities to improve dyslipidemia in rat experiments, primarily evidenced by significant reductions in serum cholesterol, triglycerides, and low-density lipoprotein levels, as well as increases in high-density lipoprotein levels in rats.

## 2. The Relationship Between Diabetes and Blood Lipids

Blood lipids refer to the total amount of lipids in plasma. Elevated blood lipid levels are a significant risk factor for cardiovascular health. Individuals with high blood lipids are at a much higher risk of developing atherosclerosis, coronary heart disease, and stroke, and they also face an increased risk of hypertension. Fat accumulation in the liver can lead to fatty liver, which may further develop into non-alcoholic steatohepatitis (NASH) and cirrhosis.

Dyslipidemia is closely related to diabetes, with over three-quarters of Type 2 diabetes mellitus (T2DM) patients experiencing abnormal blood lipid levels. This condition is

characterized by elevated triglyceride levels and reduced high-density lipoprotein cholesterol, often requiring simultaneous clinical treatment. In a hyperglycemic state, the accumulation of oxidized low-density lipoprotein (ox-LDL) increases, leading to apoptosis of insulin-secreting pancreatic beta cells, thereby triggering dyslipidemia. Insulin deficiency or insulin resistance activates intracellular hormone-sensitive lipase, increasing the release of non-esterified fatty acids from triglycerides. Moreover, diabetic patients lose the normal inhibitory effect of insulin on the production of hepatic apolipoprotein B and the secretion of very low-density lipoprotein triglycerides, resulting in increased secretion of triglyceride-rich very low-density lipoproteins. Additionally, under conditions of insulin resistance and deficiency, the content of lipoprotein lipase, which greatly determines the clearance rate of triglycerides in circulation, is downregulated.

## 3. The Effect of Steviol Glycosides on Improving Dyslipidemia in Diabetic Rats

Recent decades of experiments have shown that steviol glycosides significantly improve dyslipidemia. In intervention studies on high-fat diet-induced T2DM rats, those administered high doses of steviol glycosides exhibited a significant decrease in total cholesterol, triglycerides, and low-density lipoprotein levels, along with a notable increase in high-density lipoprotein levels. Feeding diabetic rats with rebaudioside A led to a significant reduction in serum lipoprotein levels, improving them to normal levels. Similarly, in Kurek's experiment, diabetic rats administered stevioside and rebaudioside A showed significant reductions in serum triglycerides and total cholesterol levels [2].

In a study, rats were induced with diabetes and fed a high-fat diet for eight weeks, then divided into groups and fed low and high concentrations of stevioside and rebaudioside A. Control groups included untreated diabetic rats, metformin-treated diabetic rats, and healthy rats, with the treatment lasting five weeks. The study found that the rats administered steviol glycosides restored their blood lipid levels to normal, whereas untreated diabetic rats showed a significant increase

in blood lipid levels. Additionally, the study revealed that diabetic rats administered steviol glycosides had only a slight decrease in blood adiponectin levels, but the adiponectin/leptin ratio significantly decreased. Rats fed rebaudioside A showed a significant increase in leptin levels. This indicates that steviol glycosides can improve hyperglycemia-related metabolic disorders, effectively control the appetite of diabetic rats, and thereby improve dyslipidemia.

## 4. Relevant Physiological Mechanisms

### 4.1. Steviol Glycosides Enhance Fat Metabolism

Steviol glycosides can enhance fatty acid catabolism by directly activating PPAR $\alpha$  and inducing its transcription. Activation of PPAR $\alpha$  can increase HDL levels and reduce plasma triglyceride levels, thereby improving dyslipidemia. Additionally, in diabetic conditions, stevioside and rebaudioside A affect the expression levels of lipid and glucose metabolism genes in insulin-dependent tissues such as muscle and fat. Experiments have shown that the expression of Glut4, Cebpa, and Fasn genes in the muscle tissue of diabetic rats is significantly reduced, but steviol glycosides can upregulate their expression levels<sup>[3]</sup>. Furthermore, steviol glycosides can improve glucose uptake in rat fibroblasts. This reaction is related to the activation of the PI3K/Akt pathway and the translocation of GLUT4 to the plasma membrane.

### 4.2. Steviol Glycosides Promote Insulin Secretion and Improve Insulin Resistance

Dyslipidemia is closely related to insulin levels; increasing insulin secretion and sensitivity can improve dyslipidemia. In an experiment with high-fat, diabetic male rats, five groups were administered low, medium, and high doses of stevioside, metformin, and a control group for four weeks. Results showed that high doses of stevioside significantly improved blood glucose and lipid levels in high-fat diabetic rats. High doses of stevioside stimulate insulin secretion and improve glucose tolerance. Stevioside promotes insulin secretion by directly stimulating pancreatic  $\beta$ -cells, thereby lowering blood glucose and lipid levels in rats. Additionally, the steviol glycosides' metabolite, steviol glucuronide (SVG), can promote insulin release from  $\beta$ -cells by inhibiting the transcription of B2m (an immune-related protein) and promoting the transcription and expression of the glucagon receptor (GCGR) gene<sup>[4]</sup>. Early research indicated that stevioside could improve insulin resistance in rats by inhibiting the NF- $\kappa$ B signaling pathway. When NF- $\kappa$ B is activated, it induces the expression of various pro-inflammatory cytokines, causing inflammation and reducing glucose uptake and utilization. Stevioside can also reverse established insulin resistance by inhibiting G protein-coupled receptor kinase 2 (GRK2).

### 4.3. Steviol Glycosides Promote Cholesterol Internalization

In vitro experiments studied the lipid internalization effects of steviol and stevioside, finding that high concentrations of both significantly promoted cholesterol internalization levels in HepG2 cells. Further experiments cultivated HepG2 cells with different concentrations of rebaudioside A, discovering that cells cultured with rebaudioside A showed reduced lipid

droplets and decreased low-density lipoprotein receptor (LDLR) content. The higher the concentration of rebaudioside A, the more pronounced the effect. The study also demonstrated that rebaudioside A enhances LDL cholesterol uptake in HepG2 cells by inhibiting the expression of HMGCR, LDLR, and ACAT2 genes.

### 4.4. Steviol Glycosides Have Antioxidant and Anti-inflammatory Effects

Diabetic rats exhibit significantly higher oxLDL and antioxidant enzyme GPX values compared to healthy rats, indicating increased oxidative stress due to hyperglycemia. Oxidative stress affects cholesterol metabolism pathways, increasing cholesterol synthesis in the liver and reducing the production and function of high-density lipoproteins. This leads to elevated levels of low-density lipoprotein and total cholesterol in the blood. Oxidative stress also causes lipid peroxidation, which contributes to dyslipidemia and the formation of atherosclerotic plaques. Ample experiments have demonstrated that steviol glycosides can lower oxidative stress-related markers, showcasing their antioxidant capabilities.

The interplay between oxidative stress and dyslipidemia enhances inflammatory responses. Oxidative stress-induced inflammatory factors (such as IL-6 and TNF- $\alpha$ ) can further disrupt lipid metabolism, leading to abnormal lipid levels. Studies have shown that in lipopolysaccharide-stimulated human monocyte THP1 cells, stevioside at the same concentration inhibits the release of TNF- $\alpha$ , IL-1 $\beta$ , and NO by interfering with the NF- $\kappa$ B signaling pathway. NF- $\kappa$ B is a transcription factor that controls the expression of these inflammatory cytokines in immune cells. Moreover, stevioside can prevent the adverse effects of inflammatory responses. For healthy individuals, it may provide immune-related benefits by enhancing monocyte activity.

## 5. Safety of Steviol Glycosides

### 5.1. No Genetic Toxicity

A series of in vivo and in vitro experiments on rebaudioside A have evaluated mutagenicity, chromosomal damage, and DNA strand breaks. Results indicate that rebaudioside A does not exhibit significant DNA binding or genetic toxicity. Cell-based studies have also shown that high concentrations of stevioside do not induce mutations or chromosomal breaks in bacteria or mammalian cells, both in vivo and in vitro. In 2005, the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) reported that while the metabolite steviol showed cytotoxicity in in vitro tests, stevioside itself, even at doses exceeding 2000 mg/kg body weight per day, did not exhibit significant genetic toxicity. Subsequent tests at a dose of 8000 mg/kg body weight per day also returned negative results for genetic toxicity. Notably, although steviol has shown cytotoxicity in vitro, it is rapidly excreted through urine after intestinal absorption, which, after some controversy, has been confirmed to be non-toxic.

### 5.2. No Carcinogenicity or Teratogenicity

In 1999, JECFA confirmed that steviol glycosides do not have potential carcinogenic effects. Additionally, steviol glycosides can enhance cellular phagocytic immune responses, protect cells, and exhibit anti-inflammatory and anticancer properties. Interventions with steviol glycosides in

mice with breast cancer have shown reduced tumor cell growth and increased apoptosis of tumor cells, suggesting an inhibitory effect on cancer.

Although some indigenous tribes used Stevia as a contraceptive, extensive research has demonstrated that Stevia has no contraceptive effects or teratogenicity. In studies where pregnant Wistar rats were administered stevioside daily from the sixth to the fifteenth day of pregnancy, and the rats and their fetuses were examined on the twentieth day, no toxicity or teratogenic effects were observed at doses up to 1000 mg/kg/day.

### 5.3. No Safety Concerns in Diabetic Rat Studies

Extensive evidence supports that steviol glycosides promote insulin secretion and lower blood glucose levels. However, it has been found that the insulin-promoting effect of steviol glycosides is dependent on the blood glucose concentration. Steviol glycosides enhance insulin secretion primarily when blood glucose levels are above 11.1 mmol/L. Particularly, at blood glucose levels of 16.7 mmol/L, steviol glycosides show prolonged and significantly reversible insulin-promoting effects. This indicates that steviol glycosides only promote insulin secretion at high glucose concentrations and pose no safety concerns when used in diabetic rats.

### 5.4. Historical Research and ADI

Steviol glycosides are extracts from Stevia, a traditional crop native to South America that has been used as a sweetener for centuries without reported side effects. Since the introduction of high-purity steviol glycosides to the market in 2008, no allergic reactions have been reported. In 2011, the European Union approved steviol glycosides as a food additive, and the FDA recognized them as "Generally Recognized As Safe (GRAS)" in 2018 [5]. Due to increasing evidence of their safety, JECFA further reviewed and confirmed the safety of steviol glycosides, leading to an increase in the Acceptable Daily Intake (ADI) from 0-2 mg/kg body weight per day to 0-4 mg/kg body weight per day in 2008.

## 6. Conclusion and Future Directions

Steviol glycosides are relatively safe compounds that have been shown to significantly improve dyslipidemia in diabetic

rats. As a traditional natural sweetener, steviol glycosides are considered safer and more acceptable to consumers compared to synthetic sweeteners. Current research is exploring its medicinal functions in humans, and clinical trials are gradually increasing. Future research should further investigate the long-term safety and efficacy of steviol glycosides in clinical applications. It is also important to focus on the effects and mechanisms when steviol glycosides are used in combination with other medications. Practical applications should consider the optimal dosage and usage methods to maximize health benefits. Through these in-depth studies, steviol glycosides hold promise as an adjunctive therapeutic food for improving dyslipidemia in diabetic patients, providing additional options for patients [6]. Additionally, it could offer a more solid scientific basis for the prevention and treatment of diabetes and related metabolic diseases, further advancing its use in the medical and food industries.

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