Sativeside Mitigates High-Fat Diet-Induced Inflammation and Type-2 Diabetes in Adipose Tissue of Wistar Rats

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Abstract

This study aimed to investigate the impact of Stevioside, on the biochemical changes in high-fat diet-fed Wistar rats. Adult male Wistar rats were induced into a diabetic state through the administration of a high-fat diet and sucrose for 60 days, followed by oral administration of stevioside (20 mg/kg/day) for 45 days. Various parameters, including fasting blood glucose, oral glucose tolerance, insulin, insulin tolerance, liver function (ALT, AST, ALP), kidney function (urea and creatinine), and lipid profiles (TC, TG, FFA, HDL-c and LDL-c), serum adipokines levels such as adiponectin, leptin, resistin were assessed. Stevioside treatment notably improved glucose and insulin tolerances in diabetic rats and normalized their elevated levels of fasting blood glucose, serum insulin, and lipid profile. In the high-fat diet-induced type 2 diabetes rat model, Stevioside effectively restored the altered blood serum levels, demonstrating efficacy comparable to that of metformin. Therefore, Stevioside displays promise as a potential phytomedicine for managing type 2 diabetes mellitus.

Keywords: High-fat diet, Insulin tolerance, Type-2 diabetes, Stevia rebaudiana.

Introduction

Diabetes is a metabolic disorder characterized by persistent increased levels of blood sugar, along with irregularities in blood lipids and proteins, as well as additional symptoms that elevate the risk of illness and death [1]. Diabetes poses a major challenge to public health in both the United States and globally, with its classification including type 1, type 2, and gestational diabetes [2-5]. Type 2 diabetes mellitus (T2DM) is an escalating health concern that has now reached the level of a worldwide pandemic, resulting in significant economic implications globally. Most of the global diabetes population, approximately 60%, resides in Asia, with projections indicating that this will increase further in the coming years [6].

The role of diet is pivotal in the progress of diabetes in humans. Elevated consumption of high-fat diets and the adoption of Western dietary patterns have been associated with insulin resistance and an increased susceptibility to diabetes mellitus and related metabolic disorders [7]. Currently, accessible oral hypoglycemic drugs demonstrate numerous adverse effects. Consequently, there is a demand for more potent oral antihyperglycemic agents, especially those that can restore both insulin and glucose levels to normal. A diverse range of plants and their active constituents, with minimal side effects, offer an alternative therapy for T2DM. Furthermore, the realm of plants represents a significantly underexplored source of biologically active compounds. Stevia rebaudiana is a plant species native to South America. It is well-known for its sweet-tasting leaves, which contain natural non-caloric sweeteners [8]. The sweet compounds in the leaves are called steviol glycosides, with stevioside and rebaudioside being the most...
abundant and commercially significant. Stevia has garnered attention as a natural alternative to artificial sweeteners, with a growing demand as a sugar substitute in the food and beverage industry [9]. It is several hundred times sweeter than sugar, yet it does not contribute calories or carbohydrates to the diet. This characteristic makes it a favorable option for individuals looking to reduce their calorie intake or manage conditions like obesity and diabetes. Apart from its use as a sweetener, *Stevia rebaudiana* has been explored for its potential medicinal properties [10]. Research suggests that it may have hypoglycemic [11], anti-hypertensive [12], anti-inflammatory [13], and antioxidant effects (Lemus-Mondaca et al., [14]. As a result, *Stevia rebaudiana* has attracted attention to the development of natural therapies for conditions such as diabetes, hypertension, and metabolic syndrome [15-17]. Therefore, the present study aims to determine the stability of the model induced by HFD feeding combined with sucrose water. Establishing the stability of the model will benefit the application of this model in future pharmacological studies.

**Materials and Methods**

**Chemicals and Kits**

The entire chemicals and reagents used in this research were of the molecular and analytical grade. Stevioside was purchased from Merk, Germany. Insulin ELISA kit was purchased from Krishgen Biosystems, Mumbai. Biochemical assay kits were procured from Spinreact, Spain, and Adipokinesultra-sensitive enzyme-linked immunosorbent assay (ELISA) kits were obtained from Ray Biotech.

**Experimental Design and Diabetic Induction**

Male Wistar rats weighing 170–190 g was included in this investigation. The rats were maintained under standard conditions of temperature (23°C ± 1°C) and humidity (50%–60%) on a 12-h light/dark cycle with free access to food and water. The animal study protocol was approved by the Research Ethics Committee of Saveetha Dental College, SIMATS (IAEC No: BRULAC/SDCH/SIMATS/IAEC/04-2022/107 dated 25th April 2022). The rats were randomly divided into four groups (n = 6). Group I served as the control, Group II consisted of T2DM rats, Group III comprised T2DM rats treated orally with Stevioside (20 mg/kg/day), and Group IV contained T2DM rats treated orally with metformin (50 mg/kg/day) for 45 days. Before the animals’ sacrifice, all groups underwent an Oral Glucose Tolerance test (OGTT) and FBG analysis two days earlier. Following the experimental period, the animals were euthanized, and blood samples were collected and stored at −20 °C after serum separation. Other organs were promptly excised from the rats and preserved at −80 °C for subsequent analysis.

T2DM was induced in the experimental rats through the administration of HFD consisting of 66% standard rat feed, 30% coconut oil, 3% cholesterol, and 1% cholic acid over a 60-day duration. Alongside the HFD, the rats were provided with 30% sucrose in their drinking water. To confirm the development of diabetes, the rats underwent an overnight fast on the 58th day of the experiment, following which their fasting blood glucose (FBG) levels were measured. Rats with FBG levels exceeding 120 mg/dL were identified as having T2DM and were subsequently maintained on the HFD and sucrose water until the study’s conclusion.

**Biochemical Analysis**

**Effects of Stevioside on FBG and Serum Insulin**

Following an overnight fasting period, blood samples were collected from the rat’s tail tip, and the FBG levels were evaluated using Caresens N blood glucose test strips (ISENS Biosensors India Private Limited, Gurgaon, India), with results presented in mg/dL. A commercially accessible rat insulin ELISA kit (Krishgen Biosystems, Mumbai) was employed for the quantification of serum insulin levels. The
The detection range of the kit was 0.1 to 64 ng/ml. The insulin concentration was expressed in µIU/ml.

**Stevioside Effect on LFT, KFT Markers**

Biochemical assay kits procured from Spinreact, Spain, were utilized to detect liver function markers, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), along with kidney function markers, namely, urea and creatinine. The outcomes were reported in units per liter (U/L).

**Effects of Stevioside on Lipid Profiles**

Serum triglyceride (TG), free fatty acid (FFA), total cholesterol (TC), high-density lipoproteins (HDL-c), and low-density lipoproteins (LDL-c) were analyzed using the Spinreact assay kit (Spain) according to the manufacturer's instructions. The findings were reported in milligrams per deciliter (mg/dL).

**Effects of Stevioside on Adiponectin, Leptin, Resistin**

Serum adiponectin, resistin, and leptin levels were measured using the rat insulin ELISA kit obtained from RayBiotech, USA. Adiponectin and resistin results were reported in nanograms per milliliter (ng/mL), while the leptin levels were expressed in picograms per milliliter (pg/mL).

**Statistical Analysis**

Statistical analysis was performed using a one-way analysis of variance (ANOVA), and variations between the mean values were determined using Duncan's multiple range test with GraphPad Prism version 8. Results were considered statistically significant at the p < 0.05 level.

**Results**

**Biochemical Analysis**

**Effects of Stevioside on FBG, and Serum Insulin**

To assess the potential anti-diabetic effects of Stevioside, FBG was conducted in the control and experimental animal groups. As depicted in Figure 1, the administration of Stevioside exhibited a notable reduction in elevated FBG levels in diabetic rats, comparable to the effects of metformin.

Figure 1 illustrates the serum insulin levels in the various groups of rats included in the study. Nevertheless, the Stevioside administration group showed near the normal range, thereby demonstrating its potential to enhance insulin sensitivity.
Stevioside Effect on LFT, KFT

In the diabetic group of animals, liver function markers such as ALT, AST, and ALP (Figure 2), along with renal function markers like urea and creatinine (Figure 2), were found to be elevated. Additionally, treatment with Stevioside effectively reduced these markers, demonstrating efficacy like the standard drug such as metformin.

Figure 2. Effect of Stevioside on Liver Function Markers (AST, ALP, and ALT) and Kidney Function such as Urea and Creatinine in Type-2 Diabetic Rats

Effects of Stevioside on Lipid Profiles

To assess the potential hypolipidemic effects of Stevioside, the serum levels of TC, TG, FFA, HDL-c, and LDL-c were measured in different treatment groups (Figure 3). Diabetic rats displayed signs of dyslipidemia, evidenced by a considerable increase in their serum TC, TG, FFA, and LDL-c levels, along with decreased HDL levels when compared to the control groups. However, Stevioside effectively ameliorated dyslipidemia in diabetic rats, akin to the effects of metformin, by restoring these lipid profile values to normal levels, as depicted in Figure 3.

Figure 3. Effect of Stevioside on Lipid Profiles such as TC, TG, FFA, HDL-c, and LDL-c
Effects of Stevioside on Adiponectin, Leptin, Resistin

Adiponectin, leptin, and resistin are recognized as potential serum markers of metabolic syndrome. Therefore, their levels were evaluated in the serum of both the control and experimental groups of rats. The findings indicated a notable increase (p < 0.05) in leptin and resistin concentrations, coupled with a significant decrease in adiponectin levels in diabetic rats. However, treatment with Stevioside effectively normalized the dysregulated adipokine levels in type-2 diabetic rats, showing comparable efficacy to that of metformin (Figure 4).

T2DM is a multifaceted, varied, and polygenic condition characterized by reduced insulin function (insulin resistance), along with the failure of β-cells to produce adequate insulin to counteract the insulin resistance (pancreatic β-cell dysfunction) [18]. Animal models need to emulate the phenotype and reproduce the disease's developmental process to apply to human conditions. Following 8 weeks of HFD feeding all the rats exhibited abdominal obesity, dyslipidemia, hyperglycemia, and insulin resistance, effectively replicating the natural progression of the early stages of T2DM. This serves as a crucial foundation for establishing the T2DM model [19-21].

In this study, the development of obesity by HFD in group II rats is evident by the profound increase in the FBG level. However, stevioside treatment in rats fed with HFD significantly limits the FBG level which in turn suggests that stevioside could act as a potent antiobesity drug. More importantly, after the glucose load, the blood glucose level of type 2 diabetic rats gradually increased and reached its peak value at 1 h. The elevated glucose level did not reach the normal value of 120 mg/dL even after 2 h of glucose administration, which in turn indicates glucose tolerance in these diabetic rats. However, stevioside-treated diabetic rats exhibit improved glucose tolerance as effectively as those treated with metformin. Control rats did not display any variation in glucose levels during ITT [22,23]. Likewise, insulin treatment slowly decreased the blood sugar level in type 2 diabetic rats with the minimal level being achieved only after 1 h. However, stevioside increased insulin tolerance in diabetic rats very effectively like metformin. Taken together, this evidence proves the insulin-sensitizing potential of stevioside in T2DM rats [24-26].

Stevioside effectively scavenged the surplus reactive oxygen species (ROS) generated by the
HFD, enhanced the antioxidants, and reinstated liver function in the rat model of insulin resistance caused by high fat intake. Consequently, our study findings demonstrate that Stevioside could successfully counteract oxidative stress in the gastrocnemius muscles of rats with T2DM. This study aimed to explore whether Stevioside could reverse the oxidative stress-induced reduction of insulin signaling. As anticipated, Group II rats fed with HFD and sucrose exhibited a notable increase in serum lipid profile, including FFA, TG, TC, and LDL-c, alongside a significant decrease in HDL cholesterol levels. These findings highlight the development of obesity and dyslipidemia in diabetic group rats due to excessive fat consumption [27-29]. Nonetheless, the administration of Stevioside to these diabetic rats notably improved the serum lipid profile, concurrently leading to an increase in HDL cholesterol levels, indicative of its hypocholesterolemic properties. Previous studies have reported that the application of various doses of Stevioside to diabetic rats effectively alleviated dyslipidemia [28, 30].

Insulin resistance in obese individuals primarily stems from comprehensive alterations in the metabolic and inflammatory functions of adipocytes. In the context of obesity, adipocytes tend to accumulate higher lipid levels and secrete elevated amounts of proinflammatory adipokines (such as leptin and resistin), while displaying reduced levels of anti-inflammatory adipokines (like adiponectin). These lipids and proinflammatory adipokines activate the IKKβ/NF-κB and JNK signaling pathways, subsequently stimulating the production of proinflammatory cytokines, namely TNF-α and IL-6 [31]. These cytokines facilitate the phosphorylation of serine kinases of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2), ultimately obstructing insulin signaling and glucose uptake. This represents the primary molecular and signaling mechanism underlying obesity-induced insulin resistance [20,21,32].

In the HFD and sucrose-induced type 2 diabetic rats, there was a notable elevation in serum leptin and resistin levels, alongside a significant decrease in serum adiponectin levels when compared to the control rats. Leptin, resistin, and adiponectin are crucial adipocytokines secreted by adipose tissues to communicate with various organs, including the brain, pancreas, muscle, and liver [33-36]. The current study has several limitations. Prolonged monitoring of the model and the assessment of biochemical parameters at various time points could offer additional insights, including identifying the period during which fasting blood glucose (FBG) stabilizes. Furthermore, further investigation is warranted to elucidate the underlying mechanisms contributing to the recovery observed in the model.

**Conclusion**

Our current results unequivocally demonstrate the ameliorative effects of Stevioside in the high-fat diet-induced rat model. Therefore, our study concludes that the inclusion of Stevioside as a supplement could offer a beneficial strategy for the control of T2DM. Further investigations focusing on elucidating the role of Stevioside in cell line models are necessary to establish its mechanism of action, paving the way for potential clinical trials involving this natural compound for the treatment of T2DM.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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