Anti-Diabetic Effects of *Stevia rebaudiana*

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Background

The Diabetes mellitus problem

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. It is currently described as the "Diabetes epidemic" and mostly refers to Type II diabetes mellitus (DM) or non-insulin-dependent diabetes mellitus (NIDDM)[1]. Diabetes causes mortality, weakness, and long-term complications, including an important risk factor for cardiovascular disease. It is also the leading cause of kidney failure, amputations, and blindness in adults in the West. The progression of type 2 DM begins with an impairment of glucose tolerance and is often associated with insulin resistance[2].

Many oral hypoglycemic agents currently are available along with insulin for the treatment of DM[3], but these synthetic agents can produce serious side effects, and many are not suitable for use for example during pregnancy. This leads to increasing demand for natural products having antidiabetic activity with fewer side effects, or ingredients derived from them. To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy[4]. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated.

Stevia for treating DM

Stevia rebaudiana, a perennial shrub from the Asteraceae family native to South America, is well known for its amazing sweetening properties associated with a family of glycosides

called steviosides. The main steviosides, which are calorie free, include Stevioside and Rebaudioside A which can make up more than 10% of the leaves dry weight and are over 100 times sweeter than glucose[5]. In addition, Stevia possesses a number of other phytochemicals including terpenes and flavonoids[6]. While the sweetening properties of Stevia are quite popular, the medicinal properties are less known. Stevia is used traditionally for treating diabetes as well as heart problems, obesity, and hypertension[7].

Traditional use is supported by a number of preclinical studies. In various rodent models of diabetes, extracts from Stevia leaves or purified steviosides produced anti-diabetic effects, lowering blood glucose and hepatic glycogen, while increasing insulin levels[8–12]. Moreover, clinical trials using purified steviosides verified Stevia's anti-diabetic claim with patients displaying reduced postprandial blood glucose[13].

While there are a large number of stevia based products on the market, the quality and therapeutic efficacy of the vast majority remains to be determined. This lack of verification is troubling especially when considering the known variability in plant-based products, a result of variations at a genetic, environmental, and industrial level. In this study, 2 stevia products were evaluated for their anti-diabetic activity using a cellular model of glucose uptake.

Materials and Methods

Plant material

Samples of *Stevia rebaudiana* var. "Stevi Up" including dried leaves (Sample 1) and a unique extract (Sample 2) were provided by Organi Tal. Dried samples were dissolved in water (20 mg/ml), and syringe filtered (0.2um) before being used for cellular studies.

Cell culture

L6 skeletal myoblasts (ATCC) were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS. Differentiated myotubes were produced by replacing the FBS with 1% horse serum for 5 days. MTT was utilized to verify lack of cytotoxicity in the tested samples[14].

Glucose uptake

Glucose uptake was measured based on Gorelick et al[15]. L6 cells were plated at a concentration of 10^5cells/ml on 96 well plates and differentiated. Differentiated L6

myotubes were transferred to low glucose serum free media overnight before treatment with increasing concentrations of Stevia sample (20-500ug/ml) for 4 h together with 200 uM of the fluorescent glucose analog 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG, Invitrogen). After treatment, cells were rinsed twice with cold PBS to remove free 2-NBDG and remaining fluorescence was measured with a fluorescence microplate reader (BMG Polarstar). Relative intensity minus background fluorescence was utilized for data analysis.

Statistical analysis

All data are expressed as means and standard errors. Results were analyzed by one-way analysis of variance (ANOVA), and significance differences were determined by Student's t test. Differences were statistically significant at $P < 0.05$.

Results

Glucose uptake

Both Stevia samples significantly stimulated glucose uptake in skeletal muscle cells in a dose dependent manner with a concentration of 500 ug/ml producing the greatest increase in both samples (Fig. 1).

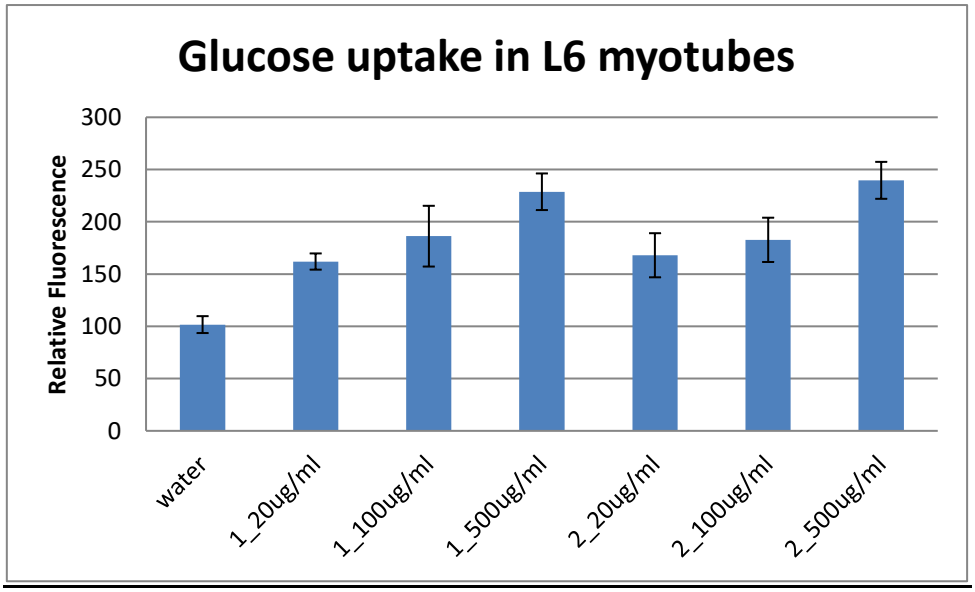


Fig. 1. Glucose Uptake in Skeletal Myotubes Treated with Increasing Concentrations of Stevia Products (1 or 2) for 4 h.

Discussion

As diabetes becomes a greater health concern, the use of alternative treatments for diabetes has become more popular. The number of herbal remedies being marketed for diabetics has increased, with nearly all pharmacies offering herbal treatments[16]. Although this increased interest has aroused the curiosity of healthcare practitioners, who have begun to document anecdotal evidence, adequate scientific studies characterizing the activity of traditional diabetic treatments are lacking. Because diabetes is a complex chronic disease involving many factors, it is difficult to develop accurate models for pharmacological testing. Since the primary symptom is elevated blood glucose due to either lack of insulin or insulin resistance, our study focused on the factors which modulate blood glucose levels, glucose uptake in skeletal muscle. These results support prior claims of Stevia's anti-diabetic activity. While Stevia's antidiabetic activity is at least partially attributed to steviosides, a number of other compounds may also be involved including flavonoids and sesquiterpenes. Further study is necessary to identify all of the active compounds responsible for Stevia's anti diabetic activity, and better understand their mode of action.

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