Research Article

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Nutritional composition of *Stevia rebaudiana*, a sweet herb, and its hypoglycaemic and hypolipidaemic effect on patients with non-insulin dependent diabetes mellitus

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Abstract

BACKGROUND: The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. India shelters the highest number of diabetics and is thus known as the 'Diabetes Capital of the World'. The chemical management of diabetes has side effects and hence the present study was undertaken to assess the hypoglycaemic and hypolipidaemic effect of *Stevia rebaudiana* in patients with type 2 diabetes, non-insulin dependent diabetes mellitus (NIDDM). Its nutritional composition and use as a sweetener substitute were also assessed.

RESULTS: Chemical analysis of dried *Stevia* leaf powder revealed it to be a nutritious herb with a good iron and fibre content. Intervention trials in diabetics revealed that it significantly lowered fasting and post-prandial blood glucose levels. The serum triglycerides and VLDL-C levels were also significantly reduced.

CONCLUSION: Hence it can be said that *Stevia* can safely be used as an anti-diabetic herb, as a sweetener substitute and may help to prevent cardiovascular diseases in patients with long-standing diabetes.

Keywords: *Stevia rebaudiana*; nutritional composition; hypoglycaemic; hypolipidaemic effect; NIDDM; anti-diabetic

INTRODUCTION

The World Health Organization (WHO) has defined diabetes mellitus as a heterogenous group of disorders characterised by a state of chronic hyperglycaemia resulting from a diversity of aetiologies. The underlying cause of diabetes is the defective production or action of insulin, a hormone that controls glucose, fat and amino acid metabolism. In diabetes the body either fails to properly respond to its own insulin, or does not make enough insulin, or both. This causes glucose to accumulate in the blood often leading to various complications.[1,2] On the basis of clinical characteristics the WHO has classified diabetes into two common types: type 1, or insulin-dependent diabetes mellitus; and type 2, or non-insulin dependent diabetes mellitus. The 2006 WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycaemia[3] are given in Table 1.

The two key features in the pathogenesis of type 2 diabetes mellitus are a decreased ability of insulin to stimulate glucose uptake in peripheral tissues, insulin resistance and the inability of the pancreatic beta cells to secrete insulin adequately, beta cell failure. The major sites of insulin resistance in type 2 diabetes are the liver, skeletal muscles and adipose tissue.[4–6]

Both defects, insulin resistance and beta cell failure, are caused by a combination of genetic and environmental factors such as life-style habits (physical inactivity, poor/faulty dietary intake), and obesity and toxins may act as initiating factors or progression factors for type 2 diabetes. The genetic factors are still poorly understood.[7–10]

Glucose control remains a major focus in the management of patients with type 2 diabetes; however, this should be in the context of a comprehensive cardiovascular risk factor reduction programme to include smoking cessation and the adoption of healthy life-style habits, blood pressure control, lipid management and, if required, platelet therapy.[11]

Glycated haemoglobin (HbA_{1c}) is a standard measure of chronic glycaemia for managing diabetes; it has been proposed as a method to diagnose diabetes and identify people at risk. In the Diabetes Prevention Programme (DPP), 3234 non-diabetic adults at a high risk of diabetes were selected and HbA_{1c} levels of more than 6.5% (48 mmol mol^{-1}) were considered to be at a high risk.[12]

Diet, exercise, modern drugs including insulin and oral administration of hypoglycaemia drugs such as sulfonylureas and biguanides manage the pathogenesis of diabetes mellitus.

Traditionally, plants and plant-derived medicines are also used for the treatment of diabetes throughout the world.[13] Management of diabetes without any side effects is still a challenge for the medical system. All artificial sweeteners like saccharin, neotame,

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Table 1. WHO diagnostic criteria for diabetes (2006)

Condition	Parameter	Diagnostic level
Diabetes	(a) Fasting plasma glucose	$\geq$7.0 mmol L^{-1} (126 mg dL^{-1})
	(b) 2 h Plasma glucose	$\geq$11.1 mmol L^{-1} (200 mg dL^{-1})
Impaired glucose tolerance (IGT)	(a) Fasting plasma glucose	<7.0 mmol L^{-1} (126 mg dL^{-1})
	(b) 2 h Plasma glucose	$\geq$7.8 and < 11.1 mmol L^{-1} (140 mg dL^{-1} and 200 mg dL^{-1})
Impaired fasting glucose	(a) Fasting plasma glucose	1–6.9 mmol L^{-1} (110–125 mg dL^{-1})
	(b) 2 h Plasma glucose	and (if measured) < 7.8 mmol L^{-1} (140 mg dL^{-1})

Source: WHO Technical Report & Recommendations 2006.[3]

Table 2. Nutrient composition of *Stevia* leaf powder

Nutrient	Amount (g kg^{-1})
Moisture	0.70 ± 0.1
Protein	0.98 ± 0.02
Fat	0.40 ± 0.06
Crude fibre	1.20 ± 0.5
Total carbohydrate	6.26 ± 0.8
Ash	0.92 ± 0.08
Iron	0.02 ± 0.01
Calcium	0.02 ± 0.01

acesulfame, aspartame and sucralose are pervasive and dangerous with several toxic side effects.

Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbology and phytotherapy.[13] *Stevia rebaudiana* is one such herb of the genus *Stevia*, widely grown for its sweet leaves. Its glycosides are stevioside and rebaudoside, which are 250–300 times sweeter than sucrose, heat stable, pH stable and non-fermentable.[14] *Stevia* has several beneficial effects, such as it helps in weight control, in the management of diabetes, control of dental caries, has anti-fungal and antibacterial properties, exerts a healing effect on blemishes and cuts, is useful in blood pressure management and aids immune modulation. It is completely safe and non-toxic. Hence the present study was undertaken to analyse the nutritional composition of *Stevia* leaf powder and to assess its hypoglycaemic and hypolipidaemic effect in subjects with type 2 diabetes mellitus. A case study approach was employed for in-depth analysis.[15]

MATERIALS AND METHODS

The study was conducted in two phases. In phase 1, chemical analysis of the nutrient composition of *Stevia* leaf powder was carried out. This included estimation of moisture content,[16] protein[16] (Microkjeldahl method), fat (solvent extraction method,[17] crude fibre (Weende's dry ashing method),[17] ash content,[16] carbohydrate,[16] calcium content (volumetric titration method)[18] and iron (spectrophotometric technique).[18]

Phase 2 included the initial screening and assessment of 20 subjects suffering from type 2 diabetes mellitus. The subjects were intensively studied for complete psycho-social and socio-economic information, medical history, anthropometric parameters which included height and weight,[19] body mass index, waist/hip ratio, mid-upper arm circumference, and biochemical estimations which included fasting and post-prandial blood glucose levels (GOD method),[20,21] triglycerides (enzymatic method),[22,23] total cholesterol,[24] LDL and VLDL cholesterol,[25] HDL cholesterol,[26] LDL/HDL ratio and atherogenic index. In addition, a detailed dietary study was done by 24 h dietary recall method for three consecutive days plus an oral questionnaire was used to collect information. The nutrients analysed were energy, carbohydrate, protein, fat and fibre.[27] The diabetic subjects were then divided into two groups of 10 subjects each. Group I served as control group and Group II subjects served as the experimental group who were given intervention with 1 g *Stevia* leaf powder which was procured from Jeevan Herbs and Agro Farms Anand (Madhya Pradesh, India)

The biochemical parameters of subjects of the control and experimental group were studied initially and then after a period of 30 and 60 days. The data collected were tabulated and analysed statistically. All the values were expressed as mean ± SD. Statistical significance was calculated at $P < 0.01$ and 0.05 using a two-tailed *t*-test.

RESULTS AND DISCUSSION

Results of the chemical analysis of the nutrient composition of *Stevia* leaf powder are given in Table 2. The results show that *Stevia* is a nutritious sweetener and could be of immense help for diabetic patients. It has a very low caloric value.[28] *Stevia* also contains two glycosides, namely stevioside and rebaudioside A. In addition to the glycosides, *Stevia* contains a diverse mixture of labdanediterpenes, triterpenes, stigmasterol, tannins as well as volatile oils.[29]

Results of dietary assessment of diabetic subjects in the control and experimental groups are presented in Table 3.

The mean daily intake of energy by male subjects was 2357 ± 417.5 Kcal by the diabetics of the control group and 2227.7 ± 370.6 Kcal by diabetics of the experimental group which was in accordance with the ICMR recommendations. This intake was lower in the case of female subjects. It was observed that the carbohydrate intake of both males and females in the control group was higher than in the experimental group. The protein intake of subjects in the experimental group was higher than in the control group. Although the carbohydrate intake was in accordance with recommended dietary allowances (RDAs) the fat intake by male subjects of the control and experimental groups was much higher as compared to the RDA. The fibre intake of both the groups (male and female subjects) was low.

The initial biochemical profile (prior to intervention) (fasting and post-prandial blood glucose levels) of subjects in the control and

Table 3. Mean intake of nutrients by male and female subjects of the control and experimental groups

	Control group ($n = 10$)		Experimental group ($n = 10$)	
Nutrient	Males ($n = 4$)	Females ($n = 6$)	Males ($n = 4$)	Females ($n = 6$)
Energy (Kcal)	2357 ± 417.55	1799.5 ± 138.88	2227.75 ± 370.66	1533 ± 262.24
Carbohydrate (g)	381.5 ± 79.5	256.33 ± 37.03	311.25 ± 29.7	215.16 ± 58.7
Protein (g)	50.5 ± 6.02	39.16 ± 7.8	75.25 ± 4.99	43.65 ± 12.45
Fat (g)	61.25 ± 10.3	38.83 ± 7.75	72.0 ± 3.55	34.16 ± 9.10
Fibre (g)	10.0 ± 2.16	10.16 ± 3.06	11.0 ± 0.81	7.55 ± 2.5

All values are mean ± SD.

Table 4. Hypoglycaemic effect [blood glucose level (mg dL^{-1})] of *Stevia* leaf powder on diabetic subjects of the control and experimental groups prior to intervention and post-intervention

	Prior to intervention		Post-intervention, 30 days		Post-intervention, 60 days	
Blood glucose	Control	Experimental	Control	Experimental	Control	Experimental
Fasting	155.62 ± 36.83	156.61 ± 31.32	161.88 ± 36.3	146.35 ± 38.17	155.29 ± 36.54	123.55 ± 22.94*
Post-prandial	232.79 ± 29.10	225.17 ± 43.86	222.67 ± 17.13	202.82 ± 45.38	228.35 ± 9.82	200.60 ± 43.80*

All values are mean ± SD.
*Significant at $P < 0.05$.

experimental groups prior to intervention and after 30 and 60 days of intervention are given in Table 4.

Subjects of the control group did not receive *Stevia* intervention while those in the experimental group received intervention. Blood glucose levels (fasting and post-prandial) were measured prior to intervention and post-intervention (after 30 and 60 days). On completion of 60 days the difference between the fasting and post-prandial blood glucose levels of diabetic subjects of the experimental group (who received intervention with *Stevia* leaves) was found to be statistically significant at $P < 0.05$ (Table 4) whereas no significant reduction in glucose levels was seen in subjects of the control group (who did not receive any intervention with *Stevia* leaves). The results are similar to other research findings which have reported a significant reduction in blood glucose levels of rats fed with stevioside, a glycoside found in *Stevia*.[30] Scientists have reported that the glycosides in *Stevia* have insulinotropic effects and may serve a potential role in the treatment of type 2 diabetes mellitus.[31]

Table 5 shows that *Stevia* leaf powder has a beneficial role in reducing serum cholesterol, triglyceride and VLDL-C levels significantly. Similar results were observed in earlier studies on lipid profile of hypertensive patients.[9]

CONCLUSION

It can be said that *Stevia* is an extremely safe and a non-toxic sweetener for diabetics and its long-term use may prove beneficial in the prevention of cardiovascular diseases in patients with long-standing diabetes. The possible glucose lowering action may be due to the direct impact of steviosides on pancreatic beta cells to secrete more insulin and to improve their function in gluco-toxicity. It can also impose its hypoglycaemic effect as it enhances the first phase insulin response and concomitantly suppresses the glucagon levels. It is thus strongly recommended for use for diabetics and may be used as a sweetener substitute as well with a great degree of efficacy and safety.

Table 5. Lipid profile of diabetic subjects of control and experimental groups prior to intervention and post-intervention

	Prior to intervention		Post-intervention, 30 days		Post-intervention, 60 days	
Lipid	Control	Experimental	Control	Experimental	Control	Experimental
Total cholesterol	175.18 ± 38.45	179.78 ± 31.58	169.85 ± 37.97	178.04 ± 0.33	172.30 ± 0.67	172.05 ± 26.20
Tryglyceride	87.36 ± 8.66	103.64 ± 36.33	79.01 ± 13.04*	72.75 ± 2.65**	79.03 ± 12.7*	70.07 ± 44.29**
HDL-C	51.26 ± 6.46	43.05 ± 7.07**	53.39 ± 4.80	44.95 ± 6.90	52.35 ± 4.92	42.97 ± 7.39
LDL-C	107.79 ± 33.76	118.22 ± 27.90	101.42 ± 35.01	118.36 ± 23.32	105.02 ± 36.90	111.63 ± 30.06
VLDL-C	17.44 ± 1.73	20.68 ± 7.27	15.77 ± 2.60*	14.49 ± 4.49**	15.78 ± 2.55*	17.96 ± 8.82**
LDL/HDL ratio	2.10 ± 00.12	2.75 ± 0.09**	1.89 ± 0.18	2.63 ± 0.96	2.0 ± 0.20	2.60 ± 0.10**
Atherogenic index	2.44 ± 0.52	3.22 ± 0.89**	2.19 ± 0.56	2.95 ± 0.94	2.30 ± 0.63	3.01 ± 0.91

Results are given as mg dL^{-1}.
All values are mean ± SD. *Significant at $P < 0.05$, **significant at $P < 0.01$.

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REFERENCES

1 Tierny LM, Mc.Phee SJ and Papadakis MA, *Current Medical Diagnosis and Treatment*, international edition. Lange Medical Books, McGraw-Hill Publishers, New York, pp. 1203–1215 (2002).
2 Rother KI, Diabetes treatment – bridging the divide. *N Engl J Med* **356**:149–150 (2007).
3 World Health Organization (WHO), *Technical Report and Recommendations: Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia*, no. 3. WHO, Geneva (2006).
4 Ostensen CG. The pathophysiology of type-2 diabetes mellitus: An overview. *Acta Physiol Scand* **171**:241–247 (2001).
5 Lindstrom J, Eriksson JG, Valle TT, Aunola S, Cepaitis Z, Hakumaki M, *et al.*, Prevention of diabetes mellitus in subjects with impaired glucose tolerance in the Finnish diabetes prevention study: Results from a randomized clinical trial. *J Am Soc Nephrol* **14**:S108–S113 (2003).
6 Holt RIG, Diagnosis, epidemiology and pathogenesis of diabetes mellitus: An update for psychiatrists. *Br J Psychiatry* **184**(Suppl. 47):s55–s63 (2004).
7 Pari L and Saravanan R Anti-diabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes Obes Metab* **6**:286–292 (2004).
8 Koski RR, Practical review of oral antihyperglycemic agents for type 2 diabetes mellitus. *Diabetes Educator* **32**:869–876 (2006).
9 Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M *et al.*, Management of hyperglycemia in type 2 diabetes: A patient centered approach: Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* **38**:140–149 (2015).
10 White JR, Davis SN, Cooppan R, Davidson MB, Mulcahy K, Manko GA *et al.*, Clarifying the role of insulin in type 2 diabetes management. *Clin Diabetes* **21**:14–21 (2003).
11 Unsitupa M, Lifestyles matter in the prevention of type 2 diabetes. *Diabetes Care* **25**:1650–1651 (2002).
12 Knowler WC, HbA_{1C} as a predictor of diabetes and as an outcome in the diabetes prevention programme: A randomized clinical trial. *Diabetes Care* **38**:51–58 (2015).
13 Acharya D and Shrivastava A, *Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices*. Avishkar Publishers and Distributors, Jaipur (2008).
14 Brandel J, FAQs – *Stevia – Nature's Natural Low Calorie Sweetner*. Agriculture and Agri Foods Canada (2004).
15 Kothari CR, *Research Methodology – Methods and Techniques*. Vishwa Prakashan, New Delhi (1990).
16 Sharma S, *Practical Biochemistry*. Classic Publishing House, Jaipur (1993).
17 Association of Analytical Chemists (AOAC), *Official Methods of Analysis*, 20th edition. AOAC, Washington, DC (1995).
18 Bureau of Indian Standards, IS 3025/1968: Methodology of Physical and Chemical Testing (1968).
19 Jelliffe DB, *The Assessment of Nutritional Status of Community*, WHO Monograph Series 53. World health Organization, Geneva (1996).
20 Trinder P, Estimation of blood glucose levels. *Am J Clin Biochem* **6**:24 (1969).
21 Samuel M and Karen SB, Modified glucose oxidase method for determination of glucose in whole blood. *Clin Chem* **19**:308–311 (1973).
22 Tietz NW, *Clinical Guide to Laboratory Tests*, 2nd edition. W.B. Saunders, Philadelphia, pp. 1073–1091 (1994).
23 Henry JB, *Clinical Diagnosis and Management by Laboratory Methods*, 18th edition. W.B. Saunders, Philadelphia, pp. 204–211 (1991).
24 Tarbutton PN and Gunter CR, Enzymatic determination of total cholesterol in serum. *Clin Chem* **20**:724–725 (1974).
25 Friedwald WT, Levy RI and Fredrickson DS, Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultra centrifuge. *Clin Chem* **18**:492–502 (1972).
26 Castelli WP, Lipids, risk factors and ischaemic heart disease. *Atherosclerosis* **124**:S1–S9 (1996).
27 Gopalan C, Ramashastri, BV and Balasubramaniam SC, *Nutritive Value of Indian Foods*, revised and updated by Rao BSN, Deosthale YG and Park KC. NIN, ICMR Hyderabad (2010).
28 Savita SM, Sheela K, Sunanda S Shankar AG, Ramakrishna P and Sakey S, Health implications of *Stevia rebaudiana*. *J Hum Ecol* **15**:191–194 (2004).
29 Sholichin M, Yamasaki K, Miyama R, Yahara S and Tanaka D, Labdane-type diterpenes from *Stevia rebaudiana*. *Phytochemistry* **19**:326–327 (1980).
30 Jeppenson PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A *et al.*, Antihyperglycemic and blood pressure reducing effects of stevioside in the diabetic goto-kakizaki rat. *Metabolism* **52**:372–378 (2003).
31 Abundula R, Matchkov VV, Jeppesen PB, Nilsson H, Aalkjaer C and Hermansen K, Rebaudioside A directly stimulates insulin secretion from pancreatic beta cells: A glucose dependent action via inhibition of ATP sensitive K^+ channels. *Diabetes, Obes Metab* **10**:1074–1085 (2008).

