

ORIGINAL RESEARCH

Effect of *Ocimum sanctum* Leaf Powder on Blood Lipoproteins, Glycated Proteins and Total Amino Acids in Patients with Non-insulin-dependent Diabetes Mellitus

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The effect of tulasi (Ocimum sanctum) powder supplementation on glycaemic control, lipidaemic control, total amino acids and uronic acid was studied in 27 non-insulin-dependent diabetes mellitus subjects. All the patients were on hypoglycaemic drugs. The above parameters were monitored at the start and on the thirtieth day. After 1 month of supplementation, a significant lowering of the blood glucose (20.8%), glycated proteins (11.2%), total amino acids (13.5%) and uronic acid (13.7%) was observed. A significant reduction was also noticed in the levels of total cholesterol (11.3%), low-density lipoprotein-cholesterol (14.0%), very low-density lipoprotein-cholesterol (16.3%) and triglycerides (16.4%). No appreciable change was noticed in high-density lipoprotein-cholesterol.

Keywords: diabetes mellitus, *Ocimum sanctum*, blood glucose, glycated proteins, lipid profile.

INTRODUCTION

The genus *Ocimum* (family Labiate) is a group of approximately 150 species of aromatic plants found mainly in the tropical and subtropical regions of the world. Many species of this genus are considered to be highly medicinal and have extensive applications in the indigenous systems of medicine in many Asian, African and South American countries {1}.

Gas liquid chromatography of the essential oil of *O. sanctum* revealed the presence of eugenol (70%) as a major constituent. The other components identified were nerol, eugenol methyl ether, caryophyllene, terpinene-4-ol, decylaldehyde, *r*-selinene, L-pinene, β -pinene, camphor and carvacrol {2}. The leaves have also been reported as yielding ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O-glucuronide, orientin and moludistin {3}.

Eugenol has been shown to inhibit lipid peroxidation efficiently {4}, the process being enhanced in the progression of atherosclerosis and in the development of secondary complications in diabetes mellitus.

O. sanctum has been reported to contain alkaloids, glycosides, tannins and saponins {5} and a number of unidentified active substances belonging to these groups, thereby indicating that the therapeutic effects of *O. sanctum* plants may be due to the presence of the above as well as a number of unidentified compounds. *O. sanctum* has also been found

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to possess an adaptogenic (anti-stress) property. It induces a state of non-specific increased resistance against a variety of stress-induced biological changes in animals {6, 7}.

A preliminary study reported a possible hypoglycaemic factor in *O. sanctum* {8}. A 50% ethanolic extract of *O. sanctum* leaves showed a hypoglycaemic effect in rats {9}. This prompted us to undertake the present study to determine the effect of the supplementation of tulasi leaves on the blood glucose, serum lipid profile, glycosylated proteins and total amino acids in non-insulin-dependent diabetes mellitus (NIDDM) subjects.

PATIENTS

Study Group

The study comprised 27 diabetics of the NIDDM type. In total, there were 17 males and 10 females. All the subjects were confirmed diabetics. The subjects were recruited from the diabetic clinic of a hospital with the consent of the consulting physician. Ninety per cent of the patients contacted agreed to join the study. Once the patients were selected, they were kept under observation for a period of 1 week before the start of supplementation. Neither drugs nor the diet were altered to any extent except for the inclusion of tulasi leaf powder during the supplementation period. Ten NIDDM patients—five males and five females—comprised the control group. They were treated in a similar fashion to the experimental group, except that they were not given any supplementation.

Supplementation

The tulasi leaves were bought from the local market. The leaves were washed thoroughly with distilled water, pressed between folds of filter paper and dried at room temperature for 2–3 days. They were then dried in an oven at 50°C for 1 h. The dried leaves were then ground to a very fine powder in a mixer. On analysis, using standard procedures {10}, 1 g of tulasi powder provides 0.20 g protein, 0.07 g fat, 0.49 g carbohydrate and 0.23 g crude fibre. The subjects were each given one packet of 1 g powder and were asked to consume it in a fasting state every morning for a period of 30 days. Compliance with the treatment was checked by periodic home visits and repeated enquiries. The packets were given for 10 days at a time and the patients were asked to visit the diabetic clinic for subsequent packet distribution and if they had any enquiries. Any patients missing doses were excluded from the study.

Chemicals

All the chemicals used were of analytical reagent grade or the best commercially available grade. Bio-gel P-6 was obtained from Bio-Rad Co., USA. All the other reagents were obtained from E. Merck, SD Fine and BDH.

Assay Methods

Measurements of the following biochemical parameters were carried out: blood glucose by Hultman's method {11}, uronic acid by Bitter and Muir's method {12}, total amino acids by Rosen's method {13}, total cholesterol using Glaxo India Ltd's enzymatic kit, triglycerides using Glaxo India Ltd's enzymatic kit and glycosylated serum proteins (GSP) by Mani *et al.*'s method {14}.

In the serum, low-density lipoprotein (LDL)-cholesterol and very low-density lipoprotein (VLDL)-cholesterol were precipitated by the addition of phosphotungstic acid and magnesium chloride {15}. The supernatant obtained was used for the determination of high-density lipoprotein (HDL)-cholesterol using Glaxo India Ltd's enzymatic kit. The

TABLE 1. Clinical data of diabetic subjects (mean \pm SD)

	Experimental group		Control group	
	Male	Female	Male	Female
Number	17	10	5	5
Age (years)	55.9 \pm 10.2	53.7 \pm 7.4	54.2 \pm 8.4	52.6 \pm 6.3
Body weight (kg)	68.7 \pm 7.2	65.0 \pm 5.6	65.4 \pm 6.6	67.7 \pm 7.2
Height (cm)	165.7 \pm 5.4	156.9 \pm 3.8	163.9 \pm 4.1	157.5 \pm 5.4
Body mass index	24.9 \pm 0.2	26.4 \pm 0.2	24.6 \pm 0.2	26.9 \pm 0.1
Duration of disease (years)	6.3	5.7	7.2	6.4

Body mass index = weight (kg)/height (m²).

Treatment: Glibenclamide, Glipizide, Penformin and Chloropropamide.

LDL-cholesterol was estimated by precipitating the serum with sodium citrate buffer (pH 5.04) containing heparin {16}. The VLDL-cholesterol was calculated by subtracting the sum of the HDL-cholesterol plus the LDL-cholesterol from the total cholesterol values.

Venous blood was collected after an overnight fast of 12–14 h and all the above parameters were estimated at the start and on the thirtieth day of supplementation.

Statistical Analysis

The mean percentage was calculated from the baseline and 30 days' supplementation data.

RESULTS

The clinical data of the diabetic patients are given in Table 1. The duration of the disease varied from 3 to 12 years in these subjects. The female diabetics had slightly higher body mass indices than the males. Tulasi powder supplementation for a period of 1 month registered a slight increase in the blood sugar and lipid levels of the controls. The experimental group exhibited a reduction in the fasting blood glucose level and in the level of glycated serum proteins. A significant reduction was also noticed in the levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglycerides after supplementation with tulasi powder. No appreciable change was noticed in the HDL-cholesterol. In addition to the blood glucose and lipid levels, the total amino acids and uronic acid also exhibited a significant reduction after 30 days of tulasi supplementation (Table 2).

DISCUSSION

The earliest recorded attempt to treat diabetes mellitus dates back more than 3500 years and the treatment used was of plant origin {17}. Plants, as folk remedies, are widely used to treat diabetes mellitus. At present, approximately 80% of the population of the Third World countries are almost dependent on traditional therapies for their health care, a fact that has been substantiated by the World Health Organization's recommendation to include traditional medicines in the primary health-care level of these countries {18}.

The present study with tulasi leaf powder in NIDDM subjects has indicated the beneficial effect of tulasi through a lowering of the blood glucose, glycated serum proteins, serum lipids, uronic acid and total amino acids.

Being a plant source, tulasi is also rich in fibre. A high fibre intake is known to be associated with a reduced incidence of diverticulosis, colon cancer, cardiovascular diseases and diabetes mellitus. It has been suggested that diabetic control may be improved upon by increasing the fibre content of the diabetic diet {19, 20}. Mani and co-workers {21–23} and

TABLE 2. Effect of tulasi powder supplementation on fasting blood sugar, uronic acid, total amino acids, glycoated serum protein, total cholesterol, lipoprotein fractions and triglyceride levels in experimental and control groups (mean \pm SD) (mmol l^{-1})

	FBS	UA	TAA	G-SP %	TC	HC	LC	VC	TG	L/H
<i>Experimental group</i>										
Baseline	10.47 \pm 4.2	3.15 \pm 0.8	4.35 \pm 0.9	2.67 \pm 0.5	5.50 \pm 1.0	1.08 \pm 0.1	3.22 \pm 0.8	1.20 \pm 0.3	2.61 \pm 0.7	3.00 \pm 0.7
After 30 days	8.15 \pm 3.3	2.66 \pm 0.4	3.69 \pm 0.6	2.37 \pm 0.4	4.86 \pm 0.8	1.05 \pm 0.1	2.76 \pm 0.7	1.02 \pm 0.3	2.22 \pm 0.8	2.63 \pm 0.7
Change in mean (%)	20.82	13.77	13.50	11.23	11.34	1.73	14.01	16.38	16.46	12.33
<i>Control group</i>										
Baseline	10.41 \pm 3.6	3.13 \pm 0.6	4.31 \pm 0.7	2.65 \pm 0.4	5.48 \pm 0.9	1.09 \pm 0.1	3.28 \pm 0.7	1.19 \pm 0.2	2.59 \pm 0.8	3.02 \pm 0.6
After 30 days	10.49 \pm 4.0	3.17 \pm 0.7	4.35 \pm 0.8	2.66 \pm 0.5	5.51 \pm 1.0	1.08 \pm 0.1	3.29 \pm 0.8	1.20 \pm 0.3	2.61 \pm 0.7	3.03 \pm 0.7
Change in mean (%)	0.76	1.27	0.92	0.37	0.54	0.91	0.30	0.84	0.77	0.33

FBS: fasting blood sugar; UA: uronic acid; TAA: total amino acids; G-SP: glycoated serum proteins; TC: total cholesterol; HC: high-density lipoprotein cholesterol; LC: low-density lipoprotein cholesterol; VC: very low-density lipoprotein cholesterol; TG: triglyceride; L/H: ratio of LC by HC.

Iyer and co-workers {24, 25} have shown that plant sources rich in fibre may not necessarily possess hypoglycaemic and/or hypolipidaemic properties, thereby suggesting the need for studying the effect of various plant sources rich in fibre.

As stated earlier, eugenol is the major component of the essential oil (70%) in tulasi leaves. Eugenol has been found to inhibit lipid peroxidation efficiently. The β -cells of the pancreas are highly susceptible to free radical damage in diabetes mellitus. It is possible that eugenol may have provided protection to the β -cells by inhibiting free-radical stress which causes an increase in insulin secretion. Tulasi leaves also contain saponins. Saponins have the capacity to form stable complexes with cholesterol and other 3- β hydroxy steroids, thereby exhibiting a cholesterol-lowering property {26}. Tulasi contains a number of other unidentified active substances. It may be said that the hypoglycaemic and hypolipidaemic activity observed may be due to the presence of the above as well as other unidentified compounds, which might have been responsible for the favourable effect of lowering the hyperglycaemia and hyperlipidaemia in NIDDM patients. It is tempting to speculate that these active compounds may have influenced the β -cells of Langerhans in enhancing insulin secretion and/or in lowering the peripheral resistance which needs further detailed investigation, with a larger sample size and longer supplementation.

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