Plant Foods for Human Nutrition **50:** 9–16, 1997.

Effect of Tulasi (*Ocimum sanctum*) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats

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Received 9 August 1996; accepted in revised form 28 August 1996

Abstract. Tulasi leaf powder was fed at the 1% level in normal and diabetic rats for a period of one month to explore the effect on fasting blood sugar, uronic acid, total amino acids, and the lipid profile in serum and tissue lipids. The results indicated a significant reduction in fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids. In liver, total cholesterol, triglyceride and total lipids were significantly lowered. Total lipids were significantly reduced in kidney. In heart, a significant fall in total cholesterol and phospholipids was observed. All these observations indicate the hypoglycemic and hypolipidemic effect of Tulasi in diabetic rats.

Key words: Diabetes mellitus, Tulasi leaves, Serum and tissue lipids

Introduction

Medicinal plants, since time immemorial have been in use for the treatment of various diseases all over the world. The genus *Ocimum* (Family-Labiatae) is a group of about 150 species of aromatic plants distributed mainly in tropical and subtropical regions of the world. Many species of this genus are considered highly medicinal and find extensive application in the indigenous system of medicine in many Asian, African and South American countries. Tulasi (*Ocimum sanctum*) or Holy Basil is an erect, hairy, annual herb, found throughout India, up to an altitude of 1,800 m in the Himalayas, which is also cultivated in temples and gardens. *O. sanctum* is rich in essential oil. Gas liquid chromatography of the essential oil has revealed the presence of eugenol (70%) as the major constituent [1]. Eugenol has been shown to efficiently inhibit lipid peroxidation [2]. Lipid oxidation has been found to occur universally in biological systems. Uncontrolled production of lipid peroxides causes a number of pathological conditions like inflammatory diseases and atherosclerosis. The association between atherosclerosis and diabetes mellitus is long known.

In addition, the essential oil from the leaves of *O. sanctum* has exhibited antibacterial and antifungal activity [1]. It has also been reported that the seeds of *O. sanctum* contain some anticoagulase factors [3]. *O. sanctum* has been reported to contain alkaloids, glycosides, tannins and saponins [4] and a number of unidentified active substances belonging to the above groups. It may be said that the therapeutic effect of *O. sanctum* plants may be due to the presence of the above as well as a number of unidentified compounds.

Being a plant sources Tulasi is rich in fiber. A high fiber intake is known to be associated with a reduced incidence of colon cancer, diverticulosis, cardiovascular disease and diabetes mellitus. It has been suggested that diabetic control may be improved by increasing the fiber content of the diabetic diet [5, 6]. Mani et al. [7–9] and Iyer et al. [10, 11] have shown that plant sources rich in fiber may not necessarily possess hypoglycemic and/or hypolipidemic properties, thereby suggesting the need for studying the effect of various plant sources rich in fiber. A possible hypoglycemic factor in *O. sanctum* has been reported in a preliminary study by Martinez et al. [12]. A 50% ethanolic extract of *O. sanctum* leaves exhibited a hypoglycemic effect in rats [13]. Because of the above factors, the objective of this project was to investigate the effect of Tulasi leaves on blood sugar and serum and tissue lipids in normal versus diabetic rats.

Table 1. Composition of Tulasi powder (g/g)

Carbohydrates	0.49	Fat	0.07
Proteins	0.02	Crude fiber	0.23

Materials and methods

Tulasi acquisition and preparation. 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 the oven at 50 °C for 1 hour. The dried leaves were then ground to a very fine powder in a mixer. This powder was incorporated into the diet. Using standard procedures [14] 1 g Tulasi powder (TP) provided 0.2 g protein, 0.07 g fat, 0.49 g carbohydrates and 0.23 g crude fiber (Table 1). The basal diet was formulated as described in Table 2. Tulasi powder was substituted at the expense of casein.

Animal preparation, housing and feeding. Thirty-two male albino rats of

the Charles Foster strain weighing between 120–140 g were divided into four groups of 8 rats each on the basis of a restricted random sampling procedure so that mean initial weights of rats in each group were comparable. Diabetes was induced in groups 3 and 4 with alloxan (Aldrich Chemical Co., USA). A dose of 100 mg/kg body weight was injected intraperitoneally in 0.5 ml of alloxan solution (prepared in saline), after 12 hours of fasting. Diabetogenic action of alloxan was checked after 7 days in urine samples for the presence of glucose, using ketodiastic reagent strips supplied by Miles India Ltd.

The alloxan induced diabetic rats were maintained on the basal diet for a period of 15 days before switching them to the different dietary regimens. The various dietary regimens (for 30 days) given to the control and experimental groups are given below and their composition is given in Table 2:

Group 1: Control rats fed basal diet.

Group 2: Control rats fed basal diet with 1% TP diet.

Group 3: Diabetic rats fed basal diet for 15 days and then continued.

Group 4: Diabetic rats fed basal diet for 15 days and then the basal diet with 1% TP diet.

The rats were caged separately and were given food and water *ad libitum*. They were sacrificed on the 30th day of the study after an overnight fast.

Table 2. Composition of experimental diets (g/100 g)

Ingredients	Basal diet	Basal + 1% TP
Casein	14.3	14.0
Vitamin mix [22]	2.0	2.0
Mineral mix [14]	4.0	4.0
Groundnut oil	5.0	5.0
Tulasi powder	—	1.0
Corn starch	74.7	74.0
Total	100.0	100.0
Calories (kcal/100g)	401.0	400.0
Protein (g/100g)	10.0	10.0

Tissue collection, preparation and assay. Tissues (blood, liver, kidney and heart) were collected at sacrifice. For sugar estimations, blood was collected in fluoride bulbs. The remaining blood sample was collected in plain tubes. Serum was separated from the blood and was used for various biochemical estimations as described below. The tissues were washed in cold saline to remove any blood, blotted on a filter paper and weighed.

The tissues were then further processed as follows: One gram of tissue (liver, kidney and heart) was suspended in 20 ml of Folch solvent [15]

(2:1, v/v; $CHCl_3$:CH_3OH) for 4–6 h, ground in mortar and pestle containing 2–3 g of Na_2SO_4. The powder thus obtained was transferred into the same Folch solvent and allowed to stand overnight. The clear supernatant was transferred into a separating funnel, mixed with an equal volume of normal saline and was allowed to stand for 4–6 h. The lower layer was collected and filtered through a Whatman No. 1 filter paper (to remove any suspended particles) rinsing the filter paper 2–3 times with Folch solvent. Filtrate and washings were then pooled. The extract was evaporated at 45–50 °C, dissolved in 1 ml of chloroform and stored at 5 °C in a tight screw-capped bottle containing a crystal of butylated hydroxytoluene as a preservative, until further analysis.

Fasting blood sugar (FBS) was determined by the method of Hultman [16]. Uronic acid was analyzed by the method of Bitter & Muir [17]. Total amino acids were assayed by the method of Rosen [18]. Total lipids were estimated by the method of Christopher et al. [19]. Triglycerides were determined using an enzymatic kit (Reckon Diagnostics, India). An enzymatic kit (Glaxo India Ltd) was used for the estimation of total cholesterol (TC). Phospholipids were analyzed by the method of Marinetti et al. [20].

In serum, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were precipitated by the addition of phosphotungstic acid and magnesium chloride [21]. The supernatant obtained was used for the determination of high density lipoprotein (HDL) cholesterol using an enzymatic kit (Glaxo India Ltd). LDL and VLDL cholesterol were calculated by subtracting HDL cholesterol value from total cholesterol.

Statistical analysis. This study involved a 2 (normal vs diabetic rats) × 2 (0 vs 1% TP) factorial design. Analysis of variance was used to determine the effect of the independent variables (type of rat and presence or absence of TP) on the various dependent variables (blood and tissue parameters). Significance was accepted at the $p \leqslant 0.05$ level.

Results

Supplementation of Tulasi powder (TP) affected the food intake and weight gain in the rats. There was a significant ($p < 0.01$) decrease in food intake and the corresponding significant ($p < 0.01$) decrease in weight gain in normal rats given 1% TP diet. There was no significant decrease in food intake in the diabetic rats regardless of the diet fed. Although a change was noted in weight gain, no change was observed in the % body weight of the organs (liver, kidney and heart) (Table 3). Diabetic rats given 1% TP diet exhibited a significant ($p < 0.01$) lowering of FBS, uronic acid and total amino acids. A significant reduction ($p < 0.01$) in TC, HDL-C, LDL+

Table 3. Effect of Tulasi powder on food intake, weight gain or loss and organ weights in rats (Mean ± SD)

Group	Food intake/day (g)	Weight gain or loss/day (g)	Liver	Kidney	Heart
			(g/100g body weight)		
Basal control	11.76 ± 1.83	3.15 ± 0.62	3.24 ± 0.18	0.65 ± 0.04	0.24 ± 0.00
Basal + 1% TP	9.74 ± 0.82**[a]	1.72 ± 0.44**[a]	3.18 ± 0.26	0.77 ± 0.02	0.24 ± 0.01
Diabetic control	18.46 ± 1.60	−0.16 ± 0.60	3.30 ± 0.30	0.72 ± 0.02	0.26 ± 0.01
Diabetic basal + 1% TP	17.41 ± 0.76	0.44 ± 1.09	3.35 ± 0.28	0.78 ± 0.14	0.26 ± 0.02

**[a] Significantly different from basal control at $p < 0.01$.

VLDL-C, phospholipids, triglycerides and total lipids was also observed in diabetic rats fed 1% TP diet (Table 4). In the liver (Table 5), TC, triglycerides and total lipids were significantly ($p < 0.01$) lowered in diabetic rats fed the Tulasi supplemented diet. A significant fall ($p < 0.01$) in total lipids in the kidney was also noted. It is interesting to note that even the control rats fed TP diet had a significant ($p < 0.05$) fall in total lipids in the kidney. In the heart, a significant ($p < 0.05$) reduction was observed in TC and phospholipids ($p < 0.01$) in diabetic rats given 1% TP diet.

Discussion

The use of plants in the therapeutic management of diabetes mellitus is gaining a lot of importance. Detailed investigations by several workers have established that plants rich in fibers have a number of beneficial effects [5, 6] particularly soluble fiber in lowering hyperglycemia and hyperlipidemia in both animal studies and in clinical trials. To date many of the components of diets consumed outside of westernized countries have not been investigated to ascertain their effect on hyperglycemia or hyperlipidemia. Therefore, the objective of this research was to determine the effect of Tulasi – a sacred herb available throughout India, on blood sugar and serum and tissue lipids in normal vs diabetic rats.

In diabetes mellitus, a primary derangement in carbohydrate metabolism along with metabolic alterations of lipid and protein contribute, to a great extent, to the development of secondary complications [23] affecting the various organs. Thus, dietary management should not only aim at normalizing

Table 4. Effect of Tulasi powder on fasting blood sugar (FBS), uronic acid, total amino acids, total cholesterol, lipoprotein cholesterol, triglycerides, phospholipids and total lipids in rats (Mean ± SD)

Groups	FBS (mg/dl)	Uronic acid (mg/dl)	Total amino acids (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL + VLDL-C (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Total lipids (mg/dl)
Basal control	70.1 ± 8.0	51.3 ± 9.6	88.8 ± 13.6	78.0 ± 12.0	48.8 ± 4.0	29.2 ± 8.9	100.9 ± 23.4	62.5 ± 15.2	276.6 ± 38.5
Basal + 1% TP	74.3 ± 8.0	41.4 ± 9.6**[a]	95.0 ± 13.6	70.6 ± 12.0	48.3 ± 4.0	22.2 ± 11.6	101.5 ± 17.2	69.6 ± 12.6	258.5 ± 45.2
Diabetic control	398.1 ± 9.6	195.5 ± 30.8	169.5 ± 14.1	172.4 ± 4.1	116.6 ± 0.6	55.8 ± 3.5	162.3 ± 8.5	99.2 ± 3.3	539.5 ± 19.5
Diabetic basal + 1%TP	172.0 ± 50.0**[b]	97.4 ± 20.5**[b]	129.5 ± 12.5**[b]	92.9 ± 3.6**[b]	50.8 ± 6.3**[b]	42.1 ± 5.0**[b]	82.2 ± 13.7**[b]	71.2 ± 10.9**[b]	294.8 ± 34.6**[b]

**[a] Significantly different from basal control at $p < 0.01$.

**[b] Significantly different from diabetic control at $p < 0.01$.

Table 5. Effect of Tulasi powder on tissue total cholesterol, triglycerides, phospholipids and total lipids in rats (Mean ± SD)

Groups	Total cholesterol (mg/dl)			Triglycerides (mg/dl)			Phospholipids (mg/dl)			Total lipids (mg/dl)		
	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart	Liver	Kidney	Heart
Basal control	1.34 ± 0.14	1.17 ± 0.17	2.90 ± 0.33	4.38 ± 2.12	2.84 ± 0.46	1.75 ± 1.12	13.53 ± 1.93	17.90 ± 1.80	20.56 ± 5.95	26.37 ± 2.07	34.02 ± 2.72	5.01 ± 1.70
Basal + 1% TP	1.43 ± 0.28	1.14 ± 0.15	2.50 ± 0.25	4.52 ± 0.77	1.13 ± 0.28	1.88 ± 0.36	11.85 ± 2.71	16.60 ± 0.53	18.02 ± 3.21	25.59 ± 1.27	31.88 ± 0.73*[a]	5.52 ± 1.83
Diabetic control	1.01 ± 0.13	1.30 ± 0.41	2.84 ± 0.05	5.90 ± 1.40	1.70 ± 1.10	1.27 ± 0.07	13.70 ± 0.20	12.30 ± 2.30	19.50 ± 2.21	24.80 ± 2.03	32.50 ± 1.03	4.06 ± 1.24
Diabetic basal + 1% TP	0.57 ± 0.14*[b]	0.79 ± 0.15	2.45 ± 0.05*[b]	3.41 ± 0.87**[b]	0.74 ± 0.50	1.24 ± 0.62	14.58 ± 2.98	11.18 ± 1.92	16.02 ± 1.12**[b]	18.73 ± 1.86**[b]	26.25 ± 1.32**[b]	4.62 ± 0.84

*[a] Significantly different from basal control at $p < 0.05$.

*[b] Significantly different from diabetic control at $p < 0.05$.

**[b] Significantly different from diabetic control at $p < 0.01$.

blood sugar level, but also normalizing serum lipid levels along with normal glycated protein levels. In this regard, detailed investigations on the influence of dietary fiber in control of diabetes mellitus are well known [24]. Besides food rich in dietary fiber, other plant foods such as spices and condiments [25] as well as others [26] have also shown the beneficial effects in reducing hyperglycemia and hyperlipidemia.

These results with Tulasi leaf powder in diabetic rats have clearly indicated the potential beneficial effects of Tulasi by bringing down the blood sugar, uronic acid, total amino acid levels, improving the lipid profile in the serum and reducing lipids in tissues. As stated earlier, *O. sanctum* contains a number of antinutrients like saponins, which could have resulted in a low food intake leading to subsequent low weight gain as noticed in control rats fed 1% TP diet. Also it is possible that *O. sanctum* may contain some active insulinogenic ingredient which helped in lowering of hyperglycemia in diabetic rats and superseding the growth depressant effect of the antinutrients, which needs further investigation. Earlier studies from this laboratory with spices [10, 11] have indicated hypoglycemic effects in human diabetics and studies by others [27], and have indicated the characterization of antioxidant principles in addition to antidiabetic principles. One of the major components of the Tulasi leaves is essential oil, eugenol, which may be assisting in exerting the hypoglycemic and hypolipidemic effect. The present observation will provide the basis for future studies with clinical trials and also in the characterization of the active principle associated with the hypoglycemic action.

Acknowledgments

The authors acknowledge the financial assistance from University Grants Commission for carrying out this work. The study forms a part of the PhD thesis work of Ms V. Rai to be submitted to MS University of Baroda.

References

1. Grover GS, Rao JT (1977) Investigations on the antimicrobial efficiency of essential oils from *Ocimum sanctum* and *Ocimum gratissimum*. Perfum Kosmet 58: 236.
2. Reddy ACP, Lokesh BR (1994) Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol Cell Biochem 137: 1–8.
3. Bhat JV, Broker R (1955) Anticoagulase factors in some indigenous plants. J Sci Ind Res 13: B: 305–306.
4. Gonopoti RD (1954) Chemical composition of *Ocimum sanctum*. Cong. Luso-espanfarm 3: 187–191.
5. Trowell H (1977) Crude fiber, dietary fiber and atherosclerosis. Atherosclerosis 16: 138–140.

6. Anderson JW(1980) The role of dietary carbohydrate and fiber in control of diabetes. Adv Intern Med 26: 67–96.
7. Mani UV, Goswamy S, Mani I, Patel S, Gujral S (1985) Effect of wheat-bran supplementation on serum lipids in diabetic rats. Arogya J Health Sci 11: 186–190.
8. Mani I, Mani UV (1987) Effect of wheat-bran supplementation on blood sugar, glycosylated protein and serum lipids in NIDDM subjects. Plant Foods Hum Nutr 37: 161–168.
9. Mani UV, Sharma M, Waghray K, Iyer U, Mani I (1989) Effect of colocasia leaves on serum and tissue lipids in cholesterol fed rats. Plant Foods Hum Nutr 39: 244–255.
10. Iyer UM, Mehta NC, Mani I, Mani UV (1992) Studies on the effect of dry Sundakai (*Solanum torvum*) powder supplementation on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. Plant Foods Hum Nutr 42: 175–182.
11. Iyer UM, Mani UV (1990) Studies on the effect of curry leaves supplementation (*Murraya koenigii*) on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. Plant Foods Hum Nutr 40: 275–282.
12. Martinez et al. (1964) A possible hypoglycemic factor in Abahaca (*Ocimum sanctum*). Ch Pb 61: 4860-d. In: Pushpangaden P, Sobti SN (1977). Medicinal properties of *Ocimum* (Tulsi) species and some recent investigation of their efficacy. Indian Drugs Aug 77: 207–208.
13. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C (1968) Screening of Indian plants for biological activity, Part I. Ind J Exp Biol 6: 232–247.
14. Raghuramulu N, Nair KM, Kalyanasundaram S (1983) A Manual of Laboratory Techniques. National Institute of Nutrition, Hyderabad, India.
15. Folch J, Lees M, Stanley GMS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509.
16. Hultman E (1959) Method of Hultman E for blood sugar. Nature 183: 108–109.
17. Bitter T, Muir H (1962) A modified uronic acid carbazole reaction. Annal Biochem 4: 330–335.
18. Rosen H (1957) Modified ninhydrin colorimetric analysis for amino acids. Arch Biochem Biophys 67: 10–15.
19. Christopher K et al. (1972) Improved determination of total serum lipids by the sulphophospho vanillin reaction. Clin Chem 18: 673–674.
20. Marinetti GV (1962) Chromatographic separation, identification and analysis of phosphatides. J Lipid Res 3: 1–4.
21. Warnick GR et al. (1982) HDL-Cholesterol quantification by phosphotungstate Mg polyethylene glycol precipitation. Both with enzymatic cholesterol assay compared with the lipid research method. Am J Clin Pathol 78: 718–722.
22. National Research Council (1972) Nutritional Requirements of the lab animals. National Academy Science Publication 277–278.
23. Truel B, Holestein CG, Fischer RN, Winternanter KH (1980) Non-enzymatic glycosylation of proteins. J Biol Chem 255: 6717–6720.
24. Jenkins DJA et al. (1976) The cholesterol lowering properties of guar and pectin. Clin Sci Molec Med 51: 8–9.
25. Sharma RD (1986) Evaluation of hypocholesterolemic factor of fenugreek seeds (*Foenum graecum*) in rats. Nutr Rep Inter 33: 669–676.
26. Sharma RD (1984) Hypocholesterolemic activity of some Indian gums. Nut Res 4: 381–389.
27. Khan BA, Abraham A, Leelamma S (1995) Hypoglycemic effect of *Murraya koenigii* and *Brasica juncea*: mechanism of action. Ind J Biochem Biophys 32: 106–108.