HYPOGLYCAEMIC, HYPOLIPIDEMIC AND ANTIOXIDANT PROPERTIES OF TULSI (*OCIMUM SANCTUM* LINN) ON STREPTOZOTOCIN INDUCED DIABETES IN RATS

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ABSTRACT

Effect of oral administration of 200 mg/Kg body weight of the aqueous extract of *Ocimum sanctum* (Tulsi) mixed with diet for eight weeks to diabetic (streptozotocin induced) rats was studied. There was significant reduction in fasting blood glucose, serum lipid profile, lipid peroxidation products, (LPO) and improvement in glucose tolerance. The aqueous extract also decreased LPO formation (thiobarbituric acid reactive substances TBARS) and increased antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GT) and one antioxidant reduced glutathione (GSH) in plasma and rat liver, lung, kidney and brain. The decrease in TBARS and increase in GSH, SOD, CAT, GPX, and GT clearly shows the antioxidant property of *Ocimum sanctum*.

KEY WORDS : *Ocimum sanctum*, diabetes mellitus, hypoglycemic effect, hypolipidemic effect, lipid peroxidation, antioxidants.

INTRODUCTION

Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for diabetes (Madhumeha). Extracts of drugs from plant sources such as *Allium sativum* (garlic), *Azadirachta indica* (neem), *Vinca rosea* (nayanantara), *Gymnema sylvestra* (meshashringa), *Trigonella foenum graecum* (fenugreek), *Momordica charantia* (bitter gourd), *Ficus bengalensis* (banyan), *Eugenia jambolana* (black berry), *Ocimum sanctum* Linn (tulsi) are some of the plants reported to possess hypoglycaemic activity in experimental animals (1-3). Tulsi is one of the aromatic plants, distributed mainly in the tropical and subtropical regions of the world including India. The species *O. sanctum* is considered to be highly sacred, medicinal and finds extensive application in the indigenous system of medicine of many Asian, African and South American countries (3,4) Tulsi is used in indigenous medicine both in Ayurveda and Siddha, in several ways (5,6). Leaves of this plant have been

used in traditional remedies to control diabetes since antiquity. Anabolic, hypotensive, smooth muscle relaxant and anti stress activity of the plant have also been reported (7,8). The leaf powder is characterized by dull green to brown colour and faint smell like that of cloves. Hypoglycemic effect of the leaf powder and leaf extract was reported in rats. (8,9). The effect of the leaves was studied in non-insulin dependent diabetes (NIDDM) patients (9). But there are no studies on the effect of tulsi on lipid peroxidation and antioxidant defence system in diabetes. In this paper, we report the effect of tulsi on the blood glucose, serum lipid peroxidation and antioxidant enzymes in normal and streptozotocin induced diabetes in rats.

MATERIALS AND METHODS

Preparation of water extract

Fresh Tulsi leaves were collected, cleaned air-dried and powdered in a grinder. 120g of air-dried powder were extracted over night with 170 ml of water with magnetic stirring. The water extract was separated and the residue was re-extracted with water. The combined water extract was concentrated in a lyophilizer. Oral administration of extract was in the

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dose of 200mg/kg-body weight (b.w).

Animals

Healthy male albino Wistar rats (200-250 gm) procured from Centre for Cellular and Molecular Biology, Hyderabad were used in this study. The animals were fed on a pellet diet (Hindustan Lever, India) and water ad libitum.

Induction of diabetes in rats

Rats were made diabetic by intraperitoneal injection of streptozotocin (STZ) 60 mg/kg in citrate buffer, pH 6.3. Five rats injected with same volume of citrate buffer acted as non-diabetic healthy controls. The rats were kept in separate metabolism cages. The diabetic rats were further divided into two groups of untreated and treated (five each). One group of untreated diabetic rats was orally administered saline daily (0.1ml/ 100 mg b. w. and the other (treated group) was orally administered daily water extract of tulsi 200 mg/Kg b.w per day in the morning for 8 weeks by bulged steel tube. The body weight was recorded weekly. At the end of the experiment animals were killed and tissues were collected and frozen in deep freezer (-4°C).

Estimations

5-ml blood was collected from the ear vein at the beginning and the end of the experiment (8 weeks). Erythrocytes and plasma were separated. Plasma glucose, total cholesterol, LDL, VLDL- and HDL-cholesterol and triglycerides were estimated as described earlier by us (10). Lipid peroxidation products were estimated as thiobarbituric acid reactive substance (TBARS) in plasma and tissues (11). Among the antioxidants, reduced glutathione was determined by the method of Hiroshi et. al. (12)

Superoxide dismutase and catalase were estimated by method of Wendel (13).

RESULTS

Oral administration of tulsi extract for 8 weeks reduced fasting plasma glucose by (35%) from the initial value of 172.5 ± 6.43 mg/dl to 112.0 ± 7.2 mg/dl. (table 1). But when compared with the diabetic untreated animals the percent reduction in plasma glucose was 62% (table 1). Improvement in glucose tolerance also was seen after tulsi treatment (table 2). For example 2 hrs after oral glucose load, the plasma glucose in untreated diabetic animals was 273.1 ± 83.2 mg/dl. But in the tulsi treated diabetic animals the blood glucose was nearly normal 74.5 ± 3.7 mg/dl. The effect of tulsi extract on the plasma

Table 1. Effect of treatment for 8 weeks with water extract of tulsi on the fasting plasma glucose level in rats.

Group	Plasma glucose mg/dl (Mean $\pm$ SD)	
	0	8 weeks
Normal	93.4 ± 24.3	83.0 ± 24.2
Diabetic untreated	165.6 ± 50.2	300.5 ± 50.7
Diabetic + tulsi	172.5 ± 6.43	112.0 ± 7.2 (-35%)

lipid profile of diabetic rats is shown in table 3. Excepting HDL cholesterol, which was unaffected, all the other plasma lipid parameters increased in diabetic untreated animals. After treatment with tulsi extract for 8 weeks, the values came back to nearly normal. In plasma and erythrocytes the antioxidant reduced glutathione and the antioxidant enzymes glutathione peroxidase and glutathione S-transferase (table 4) were decreased in diabetic animals when compared with those in controls. However, after treatment with tulsi extract for 8 weeks, there was

Table 2. Effect of treatment for 8 weeks with water extract of tulsi on plasma sugar level in glucose tolerance test (GTT) in rats.

Group	0hr.	0.5 1hr.	1hr.	1.5 hr.	2hr.
Normal	84.0 ± 6.5	150.6 ± 4.3	148.2 ± 5.6	110.5 ± 6.7	88.4 ± 5.6
Diabetic	160.7 ± 14.3	240.5 ± 77.2	270.0 ± 90.0	282.5 ± 81.4	273.1 ± 83.2
Diabetic + tulsi	82.0 ± 2.1	90.0 ± 2.7	88.3 ± 3.2	85.9 ± 4.0	74.5 ± 3.7

Table 3. Effect of treatment for 8 weeks with water extract of tulsi on lipid profile in rats.

Group	TC (mg/dl)	LDLC (mg/dl)	HDLC (mg/dl)	LDLC/ HDLC	TAG (mg/dl)
Normal	160.0 ± 14.4	82.2±8.8	46.87±8.6	1.77 ± 0.13	110.6±5.2
Diabetic untreated	235.0 ± 16.0	165.8±6.7	48.34±2.5	3.83 ± 0.19	188.1± 5.3
Diabetic+ tulsi	173.±1.24	92.5±15.6	49.2±7.3	2.0±0.4	132.0±12.6

Table 4. Effect of treatment for 8 weeks with water extract of tulsi on plasma and erythrocyte reduced glutathione, glutathione, peroxidase and glutathione transferase in diabetic rats.

Group	Reduced glutathione		Glutathione peroxidase		GlutathioneS-transferase	
	Plasma	Erythroctes	Plasma	Erythrocytes	Plasma	Erythrocytes
Control	7.36±0.53	241±17	27.08±1.9	27.67±2.0	27.08±1.9	27.67±2.0
Diabetic Untreated	5.46±0.54	148±10	28.47±3.2	21.05±1.9	26.25±1.7	15.04±10.9
Diabetic + tulsi	7.15±0.69	150±12.2	26.32±2.2	36.22±3.2	30.25±1.9	26.65±1.8

improvement in all the above parameters. In the erythrocytes, there was decrease in the levels of superoxide dismutase (SOD) and catalase (CAT) and increase in lipid peroxidation products (LPO) in diabetic rats and after treatment with water extract of tulsi there was improvement in the levels of these two antioxidant enzymes and LPO (table 5). In diabetic animals the lipid peroxidation products were elevated in the tissues which were reduced in diabetic animals treated with tulsi (table 6). There was some improvement in body weight and total hemoglobin in blood (table 7).

DISCUSSION

The results of a randomized placebo controlled,

Table 5. Effect of water extract of tulsi treatment for 8 weeks on lipid peroxidation, SOD and CAT activities in erythrocytes in rats.

Group	LPO	SOD	CAT
Control	285.9±4.5	380.4±44.3	271.8±4.1
Diabetic untreated	406.8±6.2	298.2±6.3	205.8±3.0
Diabetic + tulsi	208.4±10.5	323.6±1.5	268.3±11.0

single blind trial of holy basil (tulsi) leaves in NIDDM patients indicated a significant decrease in fasting and post prandial blood glucose levels after treatment with holy basil leaves when compared to treatment with placebo leaves (9). It is suggested that *Ocimum sanctum* potentiates the action of exogenous insulin

Table 6. Effect of treatment for 8 weeks with water extract of tulsi on lipid peroxidation products in tissues of rats.

Group	LPO (n moles /g tissue) mean ± SD				
	Plasma	Liver	Lungs	Kidney	Brain
Control	35±3.8	95.0 ± 14.3	108± 18.4	168.2± 28.0	210.4± 12.7
Diabetic untreated	68±5.4	270.0 ± 56.2	198.0±10.0	215± 60.2	263.5±51.0
Diabetic +Tulsi	25.0±1.5.	85.2 ±14.2	70.0±11.5	140.5±25.3	190.3 ±13.8

Table 7. Effect of treatment for 8 weeks with water extract of tulsi on body weight and hemoglobin.

Group	Body weight (g)	Hemoglobin
Control	180±7	14.2±3.9
Diabetic untreated	130±3.3	10.3±1.9
Diabetic +tulsi with tulsi	148±7	12.9±1.3

in normal rats (14). Recently the plant is reported to possess anti-stress or adaptogenic properties in animals and man (15). Stress is known to produce immuno suppression, and studies revealed that it has immunomodulatory activity (16). In our studies treatment of diabetic rats with tulsi extract showed considerable improvement in fasting blood glucose and in glucose tolerance also (tables 1, 2). These results conform the work of earlier workers. *Ocimum sanctum* also corrected the abnormal lipid profile seen in diabetic rats as reflected by the decrease in serum total and LDL and cholesterol level (table 3). *O. sanctum* has been shown to possess antioxidant properties in general (17). But we have carried out detailed studies on the effect of water extract of tulsi in diabetic rats. We have demonstrated for the first time that in the various tissues of diabetic rats after treatment with water extract of tulsi, there was

Increase in the level of not only the antioxidant compound reduced glutathione but also the activity of antioxidant enzymes glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase (tables 4, 5). It is now well established that oxidative stress as indicated by increase in superoxide anion and lipid peroxidation products in diabetes causes some complications (15, 16). Any drug which reduces the levels of the lipid peroxidation products in diabetics would be helpful. The elevated levels of the lipid peroxidation product (TBARS) in diabetic animals were also reduced (table 6). In conclusion water extract of tulsi possesses a wide range of beneficial effects to diabetic animals by reducing fasting blood glucose, improving glucose tolerance and serum lipid profile. The beneficial effects were seen in the various tissues of diabetic rats such as lungs, liver, kidney and brain as reflected by decrease in the lipid peroxidation products and increase in the overall antioxidant status of the animals.

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