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EVALUATION OF HYPOGLYCEMIC AND ANTIOXIDANT EFFECT OF *OCIMUM SANCTUM*

Jyoti Sethi*, Sushma Sood*, Shashi Seth and Anjana Talwar***

Department of Physiology* & Biochemistry**, Pt. B.D. Sharma, PGIMS, Rohtak

ABSTRACT

Ocimum sanctum leaves have been traditionally used in treatment of diabetes mellitus. Dietary supplementation of fresh tulsi leaves in a dose of 2 gm/kg BW for 30 days led to significant lowering of blood glucose levels in test group. Intake of *Ocimum sanctum* also led to significant increase in levels of superoxide dismutase, reduced glutathione and total thiols, but marked reduction in peroxiodised lipid levels as compared to untreated control group. The leaves were found to possess both superoxide and hydroxyl free radical scavenging action. The present observations establish the efficacy of *Ocimum sanctum* leaves in lowering blood glucose levels and antioxidant property appears to be predominantly responsible for hypoglycemic effect.

KEY WORDS

Ocimum sanctum, hypoglycemic effect, antioxidant, free radical scavenger.

INTRODUCTION

Medicinal plants, since time immemorial have been in use for treatment of various diseases all over the world. *Ocimum sanctum Linn*, (Labiatae) commonly known as "Tulsi" in Hindi is a medicinal plant commonly grown in India. The use of this herb has been reported in Indian traditional systems of medicine and its modern applications are receiving wide spread attention day by day (1, 2). Different parts of this plant have been claimed to be valuable in a wide spectrum of diseases(3). It has been observed that tulsi leaves exert hypocholes-terolemic, hypotriglyceridemic and hypophospho-lipidemic effects in the normal rabbits (4). Ethanolic extract (50%) of leaves showed hypoglycemic effects in normal as well as streptozotocin induced diabetic rats (5). Basil leaf powder has been found to cause reduction in fasting blood sugar and postprandial glucose level in NIDDM patients (6). Leaves of *Ocimum sanctum* are rich in essential oils. The presence of eugenol in it, in considerable amount has been shown to possess significant antioxidant property and to efficiently inhibit lipid peroxidation (7). In the present study, an attempt has been made to study the hypoglycemic and antioxidant effect of *Ocimum sanctum* leaves and probable link between the two effects in view of the role of reactive oxygen species in pathogenesis of Diabetes mellitus and effects of Ocimum sanctum in Diabetes mellitus.

Author for correspondence :

Dr. Anjana Talwar
C-213, Madhuvan
Delhi-110 092

MATERIAL AND METHOD

Forty albino rabbits of either sex weighing 1.5-2.5 kg were maintained under standard conditions and received food and water ad libitum. The animals were divided into two equal groups:

Control group (n=20) - Maintained on normal diet for 30 days

Test group (n=20) - Received supplementation of 2 gm fresh leaves of *Ocimum sanctum* for 30 days.

Blood samples were drawn from the central ear vein for estimation of blood glucose, plasma malondialdehyde (MDA), whole blood reduced glutathione (GSH), whole blood total thiols (PSH) and plasma superoxide dismutase (SOD) in both the groups of rabbits at the beginning of the study (day-1) and after one month (day-30) of maintenance on respective diets.

Enzymatic estimation

Plasma Malondialdehyde estimation (MDA): The lipid peroxidation products react with thiobarbituric acid forming a pink coloured adduct on boiling which was measured at 548 nm (8).

Estimation of reduced glutathione (GSH) : The protein free filtrate obtained after precipitation with metaphosphoric acid is made to react with 5,5'

dithiobis-2-nitrobenzoic acid (DTNB). This DTNB and sulphydryl groups form a relatively stable yellow colour whose absorbance is measured at 420nm against blank (9).

Estimation of plasma superoxide dismutase (SOD): Epinephrine can be autoxidized to adrenochrome by superoxide radicals. Maximum autoxidation occurs at pH 10.2 has been used as the basis for the assay of this enzyme (10).

Estimation of total thiols : 5'5' dithiobis-nitrobenzoic acid reacts with total sulphydryl groups to form a chromogen whose extinction is measured spectrophotometrically at 420 nm (11).

The data was subjected to statistical analysis using Student's paired and unpaired "t" test. A p value of less than 0.05 was accepted as indicating significant difference between the compared values

RESULTS

There was no difference in body weight gain in control and test group. Food consumption pattern in both control and test groups were comparable during the period of study. No acute or chronic adverse symptoms were observed in the test group with the dose employed. Dietary supplementation of tulsi leaves for 30 days led to decrease in blood sugar levels in test group (26%) which was highly significant statistically ($p<0.001$). Lipid peroxidation as indicated by MDA levels declined from 3.16+0.63 nmol/ml to 1.82+0.62 nmol/ml in test group. Whole blood glutathione levels in control group (0.95+0.10 mmol/l) and in test group (0.99+0.13 mmol/l) on day 1 were comparable. However, the test group showed significant increase in levels of whole blood glutathione (1.84+0.16 mmol/l) after,treatment with tulsi leaves for 30 days. There was 50.14% increase in plasma SOD levels after 30 days in the test group. Levels of whole blood total thiols were comparable in both the groups on Day 1. Dietary supplementation of tulsi leaves led to significant increase ($p<0.001$) in whole blood total thiols level in test group on Day 30.(Table I)

Table I. Effect of *Ocimum sanctum* on blood glucose, plasma malondi-aldehyde (MDA), whole blood reduced glutathione (GSH), whole blood total thiols (PSH) and plasma superoxide dismutase (SOD) (N=20)

		DAY-1	DAY-30
Blood Sugar (mg/dl)	CONTROL	150.55+ 17.94	151.07+ 17.86
	TEST	148.80+ 19.02	110.0+ 17.25**a **b
GSH (mmol/L)	CONTROL	0.95+ 0.10	0.95+ 0.11
	TEST	0.99+ 0.13	1.84+ 0.16**a **b
MDA (nmol/ml)	CONTROL	3.28+ 0.46	3.30+ 0.60
	TEST	3.16 +0.63	1.82+ 0.62**a **b
SOD (EU/ml)	CONTROL	3.68+ 0.44	3.72+ 0.85
	TEST	3.41+ 0.84	5.12+ 1.01**a *b
Total thiols (mmol/l)	CONTROL	2.94± 0.26	2.96± 0.26
	TEST	2.88± 0.33	4.16± 0.40**a **b

Values represent mean ± SD

*p< 0.001; **p< 0.01

a: statistically significant as compared to Day 1

b: statistically significant as compared to control group

DISCUSSION

Variety of Tulsi used was Sri tulsi which has green leaves and is more common. The 2gm/day dose of *Ocimum sanctum* was in accordance with the dose used by Sarkar *et al.* (2 gm fresh leaves ≃ 0.8 gm dry weight) (4). Leaves of this plant have been used in traditional remedies to control diabetes since antiquity (12). Chattopadhyay has reported a well defined role of alcoholic extract of *Ocimum sanctum* leaves in suppressing blood glucose levels in normal glucose fed hyperglycemic, insulin treated and diabetic rats as compared to control animals (5). Agrawal *et al.* (6) have suggested that basil leaves improve the B cell function and enhance insulin secretion. Sarkar *et al.* (13) have reported that on dry weight basis, leaves are apparently more effective in lowering the blood sugar levels as compared to dry seeds. Satyawati *et al.* (2) have recommended the evaluation of plant substance in the forms in which they are usually used in practice. There is a report that "tulsi" leaves inhibit absorption of glucose from the intestines, but the nature of active principle and exact mode of its action remain unclear (14). Mani *et al.* (15) have reported significant reduction in lipid profile in serum and tissue lipids in normal and diabetic rats treated with tulsi leaf powder.

Wide spread attention has been focussed on the involvement of oxygen free radicals in pathogenesis of Diabetes Mellitus (16). Cellular enzymatic (SOD) and non-enzymatic antioxidants (GSH) act as primary line of defence to cope with the deleterious effects of these radical species(17). We noticed that decreased malondialdehyde content (42.4%) with concomitant increase in both enzymatic (50.2%) and non-enzymatic (85.5%) antioxidant defence system on treatment with tulsi leaves. Kusumaran *et al.* (18) reported that intake of tulsi leaves caused a significant increase in levels of gluathione-s-transferase and all phase 1 enzymes, thus, providing protection against chemical carcinogenesis. Panda *et al.* (19) also reported significantly increased activity of two antioxidant enzymes in liver i.e. SOD and catalase following treatment with aqueous extract of *Ocimum sanctum*. Total thiols play a vital role in the structure, activity and transport function of proteins, membrane and enzymes and have been found to decrease the damage produced by oxidative stress (20). The protective effective of thiols can be brought about directly by scavenging free radicals, or indirectly by elevating GSH levels. GSH protects the cell against oxidative stress by reacting with peroxides and hydroperoxides. SOD detoxifies superoxide radicals and converts them to H_2O_2 which is further converted to H_2O by catalase or GSH peroxidase. *Ocimum sanctum* probably increased the levels of reduced glutathione in test group by facilitating reduction of oxidative free radicals by H donation. *Ocimum sanctum* probably exerted its hypoglycemic effect by increasing the glucose uptake into cell. This increased glucose level in the cell, which then via the hexose monophosphate shunt led to increased production of NADPH + H^+ and thus consequently more of reduced glutathione (GSH). Lipid peroxides in the presence of GSH are converted to alcohol derivatives and not MDA and hence in presence of increased availability of GSH (due to tulsi) MDA levels decreased to a greater extent in test group after 30 days. *Ocimum sanctum* elevated the glutathione and antioxidant enzyme levels (SOD) and decrease lipid peroxidation, thereby suggesting that hypoglycemic effect of *Ocimum sanctum* may be linked and mediated through modulation of cellular antioxidant defence system.

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