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Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats

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Abstract

Ocimum sanctum (OS) has been mentioned in Indian system of traditional medicine to be of value in the treatment of diabetes mellitus. We have previously shown that OS shows a dose-dependent hypoglycemic effect and prevented rise in plasma glucose in normal rats. It also showed significant antihyperglycemic effect in STZ-induced diabetes. The present study was undertaken to assess the effect of OS on three important enzymes of carbohydrate metabolism [glucokinase (GK) (EC 2.7.1.2), hexokinase (HK) (EC 2.7.1.1) and phosphofructokinase (PFK) (EC 2.7.1.11)] along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) in STZ (65 mg/kg) induced model of diabetes for 30 days. Administration of OS extract 200 mg/kg for 30 days led to decrease in plasma glucose levels by approximately 9.06 and 26.4% on 15th and 30th day of the experiment. Liver and two-kidney weight expressed as percentage of body weight significantly increased in diabetics ($P < 0.0005$) versus normal controls. OS significantly decreased renal ($P < 0.0005$) but not liver weight. Renal glycogen content increased by over 10 folds while hepatic and skeletal muscle glycogen content decreased by 75 and 68% in diabetic controls versus controls. OS did not affect glycogen content in any tissue. Activity of HK, GK and PFK in diabetic controls was 35, 50 and 60% of the controls and OS partially corrected this alteration.

Keywords: *Ocimum sanctum*; *Tulsi*; Holy basil; Ayurveda; Streptozotocin; Enzymes; Experimental diabetes

1. Introduction

In the recent years traditional or complementary medicine has seen an upsurge and according to two studies, almost 48.5% Australian respondents, and 34% of American respondents had used at least one form of unconventional therapy including herbal medicine (Eisenberg et al., 1993; Maclennan et al., 1996).

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics world-wide. The current treatment although provide good glycemic control but do a little in preventing complications. Besides, these drugs are associated with side effects (Rang et al., 1991). Moreover, providing modern medical healthcare across the world (especially in developing countries such as India) is still a far-reaching goal due to economic constraints. Thus, it is necessary that we continue to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be an ideal target.

Ocimum sanctum (OS) is considered a sacred plant in the Hindu culture and known as "*Tulsi*" or "Tulasi" in Hindi or Holy Basil in English. It is a tropical annual herb, up to 18 inches tall and grows into a low bush and is a member of the family Lamiaceae (Labiatae). The plant grows wild in India but is also widely cultivated in home and temple gardens. Apart from the religious significance, it also has a long history of medicinal use and is mentioned in *Charak Samhita*, the ancient textbook of *Ayurveda*. "*Indian Medicinal Plants*" Wagner et al. (1994) mention the use of Basil leaves for a variety of conditions such as catarrhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, hemorrhage, helminthiasis and topically for ring worms (Singh et al., 1980; Warier, 1995; Kirtikar and Basu, 1993). Fresh leaves taken with black pepper are used as a prophylactic measure for malaria (Dastur, 1962). It has been shown to be effective as antistress and adaptogenic and attenuates the

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stress-induced changes (Bhargava and Singh, 1981; Singh et al., 1991a,b). In addition a number of studies have shown that leaves of OS possess hypoglycemic/antihyperglycemic in experimental animals (Joglekar et al., 1959; Dhar et al., 1968; Chattopadhyay, 1993; Rai et al., 1997). In addition, a randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus has shown that the administration of plant extract significantly decreases the fasting and postprandial plasma glucose levels (Agrawal et al., 1996).

Vats et al. (2002) previously shown that single administration of 100, 200 and 400 mg/kg of alcoholic extract of OS leaves causes 7.64, 17.18 and 19.78% decrease in glucose levels in normal rats and also produce a dose-dependent reduction in plasma glucose at 60 and 120 min in OGTT conducted in rats. In addition, 3 weeks of daily oral treatment with extract in alloxanized (45 mg/kg) rats caused a significant reduction in plasma glucose levels by 11.35, 26.04, 35.72%, respectively, in rats receiving 100, 200 and 400 mg/kg of the extract. However, the duration of this study was only 21 days and the degree of hyperglycemia was only mild (200 mg/dl). Since diabetes is a chronic disorder requiring long-term therapy, we felt that there was a need to assess the effect of OS for a longer duration and in a more severe form of diabetes (plasma glucose >300 mg/dl) and for a longer duration. Moreover, no work has been reported till this date as to how the hypoglycemic and antihyperglycemic action of OS is mediated. Therefore, this study was undertaken to assess the effect of OS on three important enzymes of carbohydrate metabolism [glucokinase (GK) (EC 2.7.1.2), hexokinase (HK) (EC 2.7.1.1), and phosphofructokinase (PFK) (EC 2.7.1.11)] and glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) in STZ (65 mg/kg) induced model of diabetes for 30 days.

2. Materials and methods

2.1. Preparation and dose of extracts

Leaves of *Ocimum sanctum* were purchased from the local market and identified by Dr. E.R. Nayar of National Bureau of Plant Genetics Resources, Indian Council of Agriculture Research (NBPGR/NHCP-2002-23/666). Preliminary phytochemical evaluation of the OS leaves yielded moisture content of 84.1%, dry matter 15.9% (fiber 28%, protein 18.2%, mineral constituent 0.2%, alkaloidal content 0.21%, volatile oil 0.7%). Ethanolic extracts of OS was given in the dose of 200 mg/kg body weight.

2.2. Animals

Male albino rats (150–200 g) were obtained from the experimental animal facility of the All India Institute of Medical Sciences after obtaining approval from 'Institute Ethics Committee'. Before initiation and during the experiment, rats were fed standard chow diet. After randomization into various groups, the rats were acclimatized for 2–3 days in the new environment before initiation of experiment. Animals had free access to food and drinking water till before 30 min of sampling.

2.3. Experimental design

All the animals were randomly divided into three groups with six animals in each group. Group I (CNT) were normal and used as controls. Group II and Group III were made diabetic by a single intraperitoneal injection of Streptozotocin (65 mg/kg; Sigma, USA) and served as diabetic control and treatment groups, respectively. Rats exhibiting plasma glucose levels >300 mg/dl, 48 h after administration of STZ were included in the study. Treatment (OS extract 200 mg/kg/day orally by gavage) was started from 10th day of STZ administration.

2.4. Sample collection

2.4.1. Blood sample

It was collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in fresh vials containing sodium fluoride and sodium oxalate as anti-coagulant/anti-glycolytic agents and plasma was separated in a T8 electric centrifuger (Remi Udyog, New Delhi) at 2000 rpm for 2 min.

2.4.2. Collection of organs

After 30 days of daily feeding of extracts orally (40 days after STZ), the animals were euthanized by overdose of intraperitoneal anesthesia and blood and tissue samples were collected.

2.5. Biochemical and enzymatic estimations

2.5.1. Plasma glucose

Glucose levels were estimated by commercially available glucose kits based on glucose oxidase method (Trinder, 1969) (Autopak®, Bayer Diagnostics, Baroda).

2.5.2. Hepatic phosphofructokinase activity (EC 2.7.1.11)

The liver homogenate was prepared by grinding frozen tissue in ratio of 1:4 in 50 mM imidazole, 5 mM EDTA, 5 mM EGTA, 100 mM NaCl and 30 mM beta-mercaptoethanol (pH 7). The homogenate was centrifuged at 12,000 × *g* for 15 min and supernatant was removed and stored in ice until assay. The phosphofructokinase activity was assayed spectrophotometrically by the method of Racker (1947).

2.5.3. Hepatic glucokinase activity (EC 2.7.1.2)

The above-mentioned sample was used and enzyme activity was based on spectrophotometric method assaying glucose phosphorylation described by Pilkis (1975).

2.5.4. Skeletal hexokinase activity (EC 2.7.1.1)

The enzyme activity was determined by the method of Chou and Wilson (1975) and was based on the reduction of NADPH coupled with hexokinase measured spectrophotometrically at 340 nm.

2.5.5. Glycogen content

The tissue sample was digested in hot concentrated 30% KOH, precipitated with ethanol, hydrolyzed and finally determined as glucose in the hydrolyzate as reducing sugar (Hassid and Abraham, 1957).

2.5.6. Statistical analysis

All results are expressed as the mean ± S.D. The results were analysed for statistical significance by one-way ANOVA test using computerised software, Microcal Origin version 2.9, Northampton, USA.

3. Results

3.1. Glucose levels

Plasma glucose levels on day zero showed no significant intra-group variation. Forty-eight hours after administration of STZ, they increased by more than four folds ($P < 0.001$) while non-diabetic controls remained unchanged (Table 1). Oral administration of the extract decreased plasma glucose levels by 9.06 and 26.4% on the 15th and 30th day of the experiment, respectively ($P < 0.01$ and $P < 0.0001$, respectively).

3.2. Body weight, renal and liver weight

Table 2 shows the effect of feeding OS extract on body weight, renal and liver weight of STZ diabetic rats. Results showed no significant intra-group variation in the basal body weight. Diabetic controls did not gain any significant weight during the 30-day experimental period while normal controls and OS treated rats gained significant weight ($P < 0.0005$ and $P < 0.05$, respectively). However, the increase in treated rats was significantly lower than normal controls ($P < 0.0005$). Absolute weights of livers and kidneys were not significantly different among any of the experimental groups. When liver and two-kidney weight were expressed as percentages of body weight. There was a significant increase in diabetic rats ($P < 0.0005$) versus normal controls and this alteration in the renal weight but not in liver weight was significantly reduced ($P < 0.005$) by OS treatment.

3.3. Glycogen content

Hepatic and muscle glycogen content decreased significantly by 75 and 68%, respectively while renal glycogen content increased by over 10 folds in diabetic controls as compared to non-diabetic (Table 3). OS had no effect on glycogen content of any tissue. Brain and heart glycogen content was unchanged in all the groups.

3.4. Hepatic enzymes

The values of PFK, GK and HK decreased by approximately 40, 50 and 65%, respectively, in diabetic controls

Table 1
Effect of 30 days administration of OS extract on glucose levels (mg/dl) in STZ (65 mg/kg) diabetic rats

Group	Basal	Treatment (started on 10th day of STZ administration)		
		0 day	15th day	30th day
CNT	78.76 ± 5.38 ($n = 10$)	81.29 ± 5.44 ($n = 10$)	82.11 ± 4.81 ($n = 10$)	84.39 ± 6.77 ($n = 10$)
DCNT	77.90 ± 4.21 ($n = 10$)	325.43 ± 18.95* ($n = 8$)	318.28 ± 18.76* ($n = 7$)	321.53 ± 16.52* ($n = 6$)
OS	82.26 ± 5.74 ($n = 10$)	324.64 ± 18.31 ($n = 8$)	293.46 ± 17.78* ($n = 8$) (9.06%)	253.08 ± 31** ($n = 6$) (22.04%)

CNT: normal control; DCNT: diabetic control. Values are given as mean ± S.D. for number of animals indicated. Value in parenthesis indicates the percentage lowering of plasma glucose in comparison to the previous reading. Diabetic control was compared with normal control at the corresponding time-interval and treated group was compared with basal values at 10th day. Values are statistically significant at $^{*}P < 0.01$ and $^{**}P < 0.0001$.

Table 2
Effect of 30 days administration of OS extract on body, liver and kidney weight in STZ (65 mg/kg) diabetic rats

Groups	Body weight			Liver weight		Kidney weight	
	0 day	15th day	30th day	Absolute	LW/100 g BW	Absolute	KW/100 g BW
CNT	163.1 ± 7.88	191.4 ± 9.14	213.2 ± 6.98	7.62 ± 0.68	3.53 ± 0.25	1.54 ± 0.08	0.71 ± 0.02
DCNT	163.9 ± 10.98	165.7 ± 3.94[a,***]	168.7 ± 5.06[a,***]	7.45 ± 0.38	4.36 ± 0.16***	1.56 ± 0.04	0.92 ± 0.02***
OS	162.9 ± 9.8	170.6 ± 11.5[b,***]	181.71 ± 13.04[b,c,*,***]	7.95 ± 0.49	4.34 ± 0.11	1.60 ± 0.13	0.87 ± 0.03**

Abbreviations as for Table 1. Values are given as mean ± S.D. Value in parenthesis indicates the percentage increase or decrease vs. controls. Values are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.005$, and $^{***}P < 0.0005$.

[a] Diabetic control was compared with normal control at the corresponding time-interval.

[b] Treated group was compared with normal controls.

[c] Treated group was compared with diabetic controls.

Table 3
Effect of 30 days administration of OS extract on glycogen content (mg/g tissue) of various tissues of STZ (65 mg/kg) diabetic rats

Groups	Brain (mg/g)	Kidney (mg/g)	Heart (mg/g)	Muscle (mg/g)	Liver (mg/g)
CNT ($n = 10$)	0.40 ± 4.76	0.83 ± 0.22	1.81 ± 0.32	8.71 ± 1.22	52.55 ± 5.22
DCNT ($n = 6$)	0.43 ± 6.41	11.5 ± 2.99** (↑1280%)	1.85 ± 0.58	2.81 ± 1.26** (↓68%)	13.25 ± 3.99** (↓75%)
OS ($n = 6$)	0.41 ± 5.55	9.79 ± 2.76	1.9 ± 0.25	3.07 ± 1.26	14.5 ± 4.33

Abbreviations as for Table 1. Values are given as mean ± S.D. for number of animals indicated. Value in parenthesis indicates the percentage increase or decrease vs. controls. Diabetic control was compared with normal control at the corresponding time-interval and treated group was compared with diabetic control. Values are statistically significant at $^{**}P < 0.0005$.

Table 4
Effect of 30 days administration of OS extract on levels of three important enzymes (in percent) involved in carbohydrate metabolism in STZ (65 mg/kg) diabetic rats

Enzyme	CNT ($n = 10$)	DCNT ($n = 6$)	OS ($n = 6$)
PFK	100	59.07***	75.57***
GK	100	48.69**	63.72**
HK	100	35.22***	44.8**

Abbreviations as for Table 1. Values are given as mean ± S.D. for number of animals indicated. Value in parenthesis indicates the percentage increase or decrease vs. controls. Diabetic control was compared with normal control at the corresponding time-interval and treated group was compared with diabetic controls. Values are statistically significant at $^{**}P < 0.001$, and $^{***}P < 0.0001$.

versus normal controls and OS significantly increased PFK ($P < 0.0001$), GK ($P < 0.001$) and HK ($P < 0.001$) values (Table 4).

4. Discussion

Hypoglycemic and antihyperglycemic effects of alcoholic extract of OS in normal and chemically (alloxan 45 mg/kg) induced model of mild diabetes (plasma glucose >200 mg/dl) for 3 weeks in rodents have already been shown (Vats et al., 2002). The main goal of the present study was to (a) determine the antihyperglycemic effect of alcoholic OS extract in moderate form of diabetes (plasma glucose >300 mg/dl), (b) to study this effect for longer duration, i.e. 4 weeks, (c) to ascertain if OS modulates glycogen content of insulin-dependent (skeletal muscle and liver) and -independent tissues (kidneys and brain), and lastly (d) to assess the effect of OS on the three key enzymes involved in carbohydrate metabolism (PFK, GK, HK). The present study is important, as this is the first biochemical inspection of the effects of OS on the gluconeogenic enzymes and glycogen content in experimental type-1 diabetes.

Feeding of OS extract (200 mg/kg) for 30 days failed to achieve euglycemia but caused a significant ($P < 0.01$) reduction in glucose levels by 10% in comparison with the basal levels. Moreover, a 10% reduction in plasma glucose levels in this study is much lesser than reported in our previous study where almost 25% reduction in glucose level was achieved in alloxan (45 mg/kg) induced diabetes. It has been shown that the destruction of β-cells of the pancreas is directly proportional to the dose of the diabetogenic agent (Junod et al., 1969). Since antihyperglycemic response of OS is different in the two models (alloxan-45 mg/kg and STZ 65 mg/kg) of varying intensity of hyperglycemia (plasma glucose >200 and >300 mg/dl, respectively), it implies that the antihyperglycemic effect of OS is at least partially dependent upon insulin release from the pancreas, as the extract showed a greater antihyperglycemic effect in milder form of diabetes but a lower response in moderate form of diabetes. Such a response has also been seen previously with extracts of *Eugenia jambolana* (Grover et al., 2000) and *Momordica charantia* (Rathi et al., 2002).

During the experimental period the normal controls showed an approximately 30% gain in the body weight. On the other hand diabetic controls showed no significant gain in body weight over the same time period. Failure to gain body weight in animals administered STZ has already been reported (Chen and Ianuzzo, 1982; Raju et al., 2001). OS treated rats showed higher and significant gain in the body weight in comparison to diabetic controls but was lower than in the normal controls.

STZ-induced diabetic animals tend to show renal hypertrophy. The entry of glucose in renal tissue is not dependent on action of insulin and, therefore, in the event of hyperglycemia there is an increase in the entry of glucose (Belfiore et al., 1986). This has been postulated to cause increased intra-renal glycogen deposition, which leads to glycosylation of basement membrane collagen in the kidney (Anderson and Stowring, 1973). This is reflected in present finding as the diabetic rats showed a 29% increase in two-kidney versus body weight ratio in comparison to controls as well as a 13 fold increase in renal glycogen content. Rise in renal weight as well as renal glycogen content has been reported previously (Anderson and Stowring, 1973) though in previous studies (Rasch, 1980; Nielsen et al., 1999) the degree of renal hypertrophy was much higher in comparison with the present study. The reason for this disparity in the present and the past results can be attributed to the difference in the duration of the studies, i.e. 1 month versus 6 and 2 months, respectively. OS significantly lowered this renal hypertrophy but could not normalise it (Table 2). This may be because of failure of OS to achieve euglycemia. Brain and heart glycogen content remained unaltered in the experimental groups and this is in agreement with our earlier findings (Grover et al., 2000; Rathi et al., 2002).

The literature regarding the effect of diabetes on liver weight is contradictory as some workers have shown an increase in hepatic weight in animals (Chen and Ianuzzo, 1982; Sadique et al., 1987; Murphy and Anderson, 1974) as well as humans (Van Lancker, 1976) while others have reported no change (Gupta et al., 1999). In the present case diabetic rats showed significantly higher liver weight/100 g body weight and this ratio was not altered by OS. Exact reasons of hepatic hypertrophy are not known, however fat deposition has been proposed to be the cause (Sadique et al., 1987).

Activities of GK and PFK have been shown to be very sensitive signs of the glycolytic pathway (Murphy and Anderson, 1974) and this is decreased in diabetic state (Grover et al., 2000; Rathi et al., 2002). Administration of insulin causes normalisation of the enzymatic activities (Weber et al., 1966) and, therefore, measurement of the activity of these enzymes represents a method to assess peripheral utilisation of glucose. In the present study levels of glycolytic enzymes in liver and skeletal muscle were decreased in the diabetic controls. This reaffirms our previous findings (Grover et al., 2000; Rathi et al., 2002) as well as those of others (Raju et al., 2001) that relative deficiency of insulin in chemical model of Type I diabetes causes suppression of the PFK, HK and GK activities. Maximum suppression was seen in the skeletal HK activity followed by hepatic GK and PFK activity. Administration of OS extract increased significantly the activity of all the three enzymes towards normal controls suggesting that the antihyperglycemic action seen is the result of an increased glucose utilisation at the level of skeletal muscle as well as liver. However, it is not possible to deduce from the present finding that the increase in glycolytic enzymatic activity seen in the OS fed rats occurred secondary to the OS mediated release of insulin or whether a component of OS had insulinomimetic action. Since STZ diabetes is an insulin deficient model, the probability of insulinomimetic effect seems more probable.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ causes selective destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose (Whitton and Hems, 1975; Golden et al., 1979; Bishop, 1970). Moreover, this alteration in muscle and hepatic glycogen content is normalised by insulin treatment (Weber et al., 1966; Steiner and King, 1964). Results showed that hepatic and skeletal glycogen content decreased drastically in diabetic controls by almost 3/4th of their basal levels. This has also been reported earlier (Hikino et al., 1989). OS showed a trend towards an increase in glycogen content but the effect was not significant, even though it significantly raised the levels of HK and GK. Since the OS extract prevented the loss of body weight (or catabolism) seen in diabetic controls, it is possible that it may have increased the glycogen content in muscle and liver but the same was utilised for energy expenditure instead of being stored.

The current study provides some useful insight into the molecular effect of feeding alcoholic extract of OS. Variety of studies using different animals models of stress suggest that OS can ameliorate physiological responses to stress there by enabling the body to cope with stress. A number of studies have also demonstrated that sacred basil possesses good antioxidant activity (Shyamala and Devaki, 1996; Ganasoundari et al., 1997a,b; Ganasoundari et al., 1998). Thus, the antihyperglycemic activity of OS supplemented with its adaptogenic and anti-oxidant activity will be an ideal multi-pronged treatment for managing diabetes, as it will target the stress, catabolism and glycemia associated with the disease. Moreover, the safety of OS is evident from the long history of safe traditional uses in India (Wagner et al., 1994) and its high LD_{50} value (4508 ± 80 mg of ethanolic extract in mice). Though anti-fertility including abortifacient and anti-spermatogenic effects have been described (Seth et al., 1981; Khanna et al., 1986), these occurred at very high doses (equivalent to a daily dose of 50 g or more in humans). Thus, *Ocimum sanctum* needs clinical evaluation before being named as an antidiabetic herb.

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