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Effect of *Scoparia dulcis* (Sweet Broomweed) plant extract on plasma antioxidants in streptozotocin-induced experimental diabetes in male albino Wistar rats

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Clinical research has confirmed the efficacy of several plants in the modulation of oxidative stress associated with diabetes mellitus. *Scoparia dulcis* plant extract is tried for prevention and treatment of diabetes mellitus induced experimentally by streptozotocin injection. A single dose of streptozotocin (45 mg/kg body weight) produced decrease in insulin, hyperglycemia, increased lipid peroxidation (Thiobarbituric reactive substances and lipid hydroperoxides) and decreased antioxidant levels (vitamin C, vitamin E, reduced glutathione, ceruloplasmin). Oral administration of an aqueous extract of *Scoparia dulcis* plant (200 mg/kg body weight) for 6 weeks to diabetic rats significantly increased the plasma insulin and plasma antioxidants and significantly decreased lipid peroxidation. The effect of *Scoparia dulcis* plant extract at 200 mg/kg body weight was better than that of glibenclamide, a reference drug.

1. Introduction

Chronic hyperglycemia is the primer of a series of cascade-reactions causing the overproduction of free radicals, and increasing evidence indicates that these contribute to the development of diabetic complications (Aragno et al. 1999). On the other hand, our bodies possess some defenses against oxidative stress via endogenous or exogenous antioxidative substances that are markedly altered during diabetes (Furusho et al. 2002).

Long established systems of traditional medicine have evolved from systematic recordings of human experience over several millennia (Hu et al. 2003). *Scoparia dulcis,* a traditional Indian medicine herb, is considered as a useful medicine for the amelioration of diabetes (Nath 1943). It has been reported that *Scoparia dulcis* has traditionally been used as one of remedies for stomach troubles (Satyanarayana 1969), hypertension (Chow et al. 1974), diabetes (Perry 1980), inflammation (Gonzales Torres 1986), bronchitis (Farias Freie et al. 1993) hemorrhoids and hepatosis (Satyanarayana 1969) and as an analgesic and antipyretic (Gonzales Torres 1986). The active principles are scoparic acid A, scoparic acid B (Hayashi et al. 1993), scopadulcic acid A and B, scopadulciol (Hayashi et al. 1990) and scopadulin (Hayashi et al. 1991). These compounds were found to possess various biological activities for example as inhibitors against replication of herpes simplex virus, as gastric H^+, K^+ ATPase activators and in antitumor promoting. Nath (1943) studied the antidiabetic effect of *Scoparia dulcis* and obtained a glycoside, almelin, from fresh plant and reported that it brought relief in ailments accompanied with diabetes (ie., pyorrhoea, eye troubles, joint pain, susceptibility to cold etc.) within a very short period.

The present study was aimed at determining the influence of an aqueous extract of *Scoparia dulcis* (SPEt) on lipid peroxidation and the levels of antioxidants in plasma of rats treated with streptozotocin.

2. Investigations and results

2.1. Effect of Scoparia dulcis plant extract (SPEt) on blood glucose and plasma insulin

In all groups or rats, the basal levels of blood glucose of the rats were not significantly different prior to streptozotocin administration. However, 48 h after streptozotocin administration, blood glucose levels were significantly higher in rats selected for the study (Table 1). In contrast, non-diabetic controls remained persistently euglycaemic throughout the course of the study.

Table 1 shows the effect of treatment with extracts on blood glucose levels. In all the SPEt treated groups (50, 100 and 200 mg/kg), although a significant antihyperglycaemic ($p < 0.01$) effect was evident from the first week onwards, decrease in blood sugar was maximum on completion of the sixth week (65.63%) ($p < 0.001$) in the group receiving 200 mg/kg/day of aqueous extract of *Scoparia dulcis*. On the basis of these studies, a dose of 200 mg/kg aqueous extract of *Scoparia dulcis* per day was selected for further evaluation. Administration of SPEt and glibenclamide to diabetic rats significantly increased the level of plasma insulin.

2.2. Effect of SPEt on plasma lipid peroxidation and antioxidants

Lipid peroxidative markers namely: thiobarbituric acid reactive substances (TBARS) and hydroperoxides (Table 2)

Table 1: Effect of 6-weeks treatment with various doses of aqueous extract of *Scoparia dulcis* on glucose levels in normal and experimental rats

Groups	'0' day	48 h after STZ injection	I week (after treatment)	II week	III week	IV week	V week	VI week
	Blood glucose (mg/dl)							
Normal	84.02 ± 6.99	85.33 ± 5.92	82.16 ± 5.92	84.57 ± 6.16	83.50 ± 6.37	81.40 ± 7.65	82.75 ± 5.64	83.07 ± 6.82
Normal + SPEt-Aq-200	83.66 ± 6.59	82.25 ± 4.66	80.50 ± 5.56	78.50 ± 4.30	74.35 ± 4.99	72.48 ± 5.76	73.25 ± 5.91	71.62 ± 4.78
Diabetic control	78.80 ± 4.30	253.80 ± 16.20**	263.56 ± 20.80**	279.86 ± 12.93**	301.01 ± 12.40**	319.03 ± 15.28**	329.12 ± 17.77**	336.54 ± 13.40**
Diabetic + SPEt-Aq-50	77.36 ± 4.24	241.68 ± 14.63**	219.62 ± 12.20* (9.12)	199.56 ± 10.21** (17.42)	179.35 ± 11.99** (25.79)	159.33 ± 7.21** (34.07)	131.60 ± 6.67** (45.52)	108.28 ± 6.86** (55.19)
Diabetic + SPEt-Aq-100	79.01 ± 4.50	248.00 ± 14.02**	220.19 ± 11.84* (11.21)	197.48 ± 9.31** (20.37)	162.58 ± 6.86** (34.44)	140.30 ± 5.19** (42.45)	118.16 ± 6.43** (52.35)	98.50 ± 4.89** (60.28)
Diabetic + SPEt-Aq-200	79.66 ± 3.03	256.60 ± 16.80**	214.91 ± 11.35** (16.24)	175.91 ± 7.15** (31.44)	113.00 ± 8.48** (55.96)	106.20 ± 7.01** (58.61)	93.59 ± 6.75** (63.52)	88.19 ± 6.40** (65.63)
Diabetic + Glibenclamide (600 μg/kg)	77.88 ± 4.48	245.58 ± 13.99**	219.29 ± 7.05* (10.70)	191.58 ± 10.8** (21.98)	118.22 ± 4.48** (51.86)	110.69 ± 6.27** (54.92)	97.77 ± 6.18** (60.18)	89.80 ± 6.32** (63.42)

Values are given as mean ± S.D for 6 rats in each group. Values in parentheses indicated the percentage lowering of blood glucose in comparison to basal reading after streptozotocin (STZ) administration at 48 h. Diabetic control was compared with normal. Experimental groups were compared with corresponding values after streptozotocin injection (48 h). * $-p < 0.01$, ** $-p < 0.001$

from plasma of diabetic rats were significantly increased and antioxidants: vitamin C, vitamin E, reduced glutathione and ceruloplasmin were significantly decreased. Treatment with SPEt and glibenclamide significantly decreased the levels of lipid peroxidation products and increased the plasma antioxidant levels.

3. Discussion

The involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defense system have been studied. For the study of antidiabetic agents, streptozotocin induced hyperglycemia in rodents is considered to be good preliminary screening diabetic model (Ivorra 1989) and is widely used. Streptozotocin, *N*-{methylnitrocarbamoyl}-D-glucosamine is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cells. β-Cells are particularly sensitive to damage by nitric oxide and free radical because of their low levels of free radical scavenging enzymes (Lukic 1998). Streptozotocin directly generates oxygen free radical induced lipid peroxidation (Spinas 1999; Bassirat and Khalil 2000). This study was therefore undertaken to assess antiperoxidative properties of *Scoparia dulcis* in streptozotocin diabetic rats.

In the present investigation, treatment with an aqueous extract of *Scoparia dulcis* showed significant antihyperglycaemic activity. The maximum reduction in glucose levels was seen in animals receiving 200 mg/kg of the extract. This probably indicates an efficacy of the plant. Moreover, it seems that the antihyperglycaemic activity of this plant is partly due to release of insulin from existing β-cells of the pancreas. The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycaemic sulphonylureas, which promote insulin secretion by closure of K^+-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. In this context a number of other plants have also been reported to have antihyperglycaemic and insulin-release stimulatory effects (Venkateswaran and Pari 2002; Latha and Pari 2003).

It has been generally reported that diabetic patients with vascular lesions have higher TBARS levels than their healthy counterpart. TBARS and hydroperoxides are significantly increased in diabetic rats. Previous studies have shown that there was an increased lipid peroxidation in plasma of diabetic rats (Prince and Menon 1998; Venkateswaran et al. 2002). Our study shows that administration of SPEt to diabetic rats tends to bring the plasma peroxides to near normal levels.

Ascorbic acid is a potent antioxidant, which widely acts on oxygen free radicals (OFR) as well as by interaction with vitamin E (Garg and Bansal 2000). Both the vitamins C and E significantly are decreased in the plasma of diabetic rats. Administration of SPEt increased the vitamin C and E levels. This indicates that vitamin E is used in combating free radicals and if vitamin C is present, vitamin E levels are preserved. Frei (1991) has previously shown the ability of vitamin C to preserve the levels of other antioxidants in human plasma. Also vitamin C regenerates vitamin E from its oxidized form. GSH is the first line of defense against a proxidant status (Ahmed et al. 2000) and GSH was evaluated after SPEt administration. GSH systems may have the ability to manage oxidative stress with adaptional changes in enzymes regulating GSH metabolism. In the present study, treatment with SPEt significantly increased GSH levels. Increase in GSH levels may in turn activate the GSH

Table 2: Changes in levels of TBARS, hydroperoxides, vitamin C, vitamin E, reduced glutathione (GSH) and ceruloplasmin in plasma of normal and experimental animals

Groups	Normal	Normal+SPEt-Aq-200	Diabetic control	Diabetic+SPEt-Aq-200	Diabetic + Glibenclamide (600 μg/kg)
Plasma insulin (μU/ml)	10.38 ± 0.94^a	11.04 ± 0.72^a	4.20 ± 0.04^b	9.28 ± 0.40^c	8.52 ± 0.42^c
TBARS (mM/dl)	0.17 ± 0.01^a	0.15 ± 0.04^a	0.40 ± 0.03^b	0.20 ± 0.01^c	0.26 ± 0.02^d
Hydroperoxides ($\times 10^{-5}$ mM/dl)	10.08 ± 0.61^a	9.00 ± 0.58^a	20.00 ± 1.29^b	12.33 ± 0.47^c	13.13 ± 0.48^c
Vitamin C (mg/dl)	1.68 ± 0.07^a	1.88 ± 0.05^b	0.75 ± 0.04^c	1.59 ± 0.06^d	1.44 ± 0.05^e
Vitamin E (mg/dl)	1.52 ± 0.07^a	1.74 ± 0.06^b	0.68 ± 0.02^c	1.35 ± 0.06^d	1.28 ± 0.05^e
Ceruloplasmin (mg/dl)	18.50 ± 1.70^a	16.43 ± 1.07^b	30.80 ± 2.32^c	21.53 ± 1.54^d	24.35 ± 1.46^e
Reduced Glutathione (mg/dl)	25.23 ± 2.50^a	28.16 ± 1.52^b	12.00 ± 0.46^c	21.25 ± 1.36^d	19.05 ± 1.26^d

Values are given as mean ± S.D for 6 rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan procedure, Range for the level 2.91, 3.06, 3.16, 3.22

dependent enzymes such as glutathione peroxidase and glutathione-S-transferase.
Ceruloplasmin is an important enzyme, which oxidizes iron from the ferrous to the ferric state and it has been demonstrated that iron catalysed lipid peroxidation requires both Fe(II) and Fe(III) and the maximum rate occurs when the ratio is approximately one (Bucher et al. 1983). The level of ceruloplasmin is reported to increase under conditions leading to the generation of oxygen products such as superoxide radical and hydrogen peroxide (Dormandy 1980). The observed increase in the level of plasma ceruloplasmin in diabetic rats may be due to increased lipid peroxides. Prince and Menon (1999) also reported an increased level of ceruloplasmin in diabetic rats. Administration of SPEt to diabetic rats restored the level of ceruloplasmin to near normal level.
Hence, in addition to the antidiabetic effect, *Scoparia dulcis* possesses antioxidant potential that may be used for therapeutic purposes. The results of the present study indicate that the preventive effects of *Scoparia dulcis* may be due to an inhibition of lipid peroxidation. However, more mechanistic studies are essential to elucidate the exact mechanism of this modulatory effect.

4. Experimental

4.1. Animals

Male albino Wistar rats, body weight of 180–200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The animals were fed on a pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the ethical committee (Vide. No: 73, 2002), Annamalai University.

4.2. Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade.

4.3. Plant material

Scoparia dulcis L. plants were collected from Neyveli, Cuddalore District, Tamil Nadu, India. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No. 3412) was deposited in the Botany Department of Annamalai University.

4.4. Preparation of plant extract

500 g of *Scoparia dulcis* fresh whole plants were extracted with 1.5 L of water by the method of continuous hot extraction. The filtrate was evaporated to constant weight on a rotavapor. The residual extract was dissolved in sterile water and used in the investigation (Jain 1968).

4.5. Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg (Siddique et al. 1987). 48 h after streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycaemia (i.e with blood glucose of 200–300 mg/dl) were taken for the experiment.

4.6. Experimental design

In the experiment, a total of 42 rats (30 diabetic surviving rats, 12 normal rats) were used. The rats were divided into 7 groups of 6 rats each. Group 1: Normal rats. Group 2: Normal rats given *Scoparia dulcis* plant extract (SPEt) (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 6 weeks. Group 3: Diabetic control rats. Group 4: Diabetic rats given SPEt (50 mg/kg body weight) in aqueous solution daily using an intragastric tube for 6 weeks. Group 5: Diabetic rats given SPEt (100 mg/kg body weight) in aqueous solution daily using an intragastric tube for 6 weeks. Group 6: Diabetic rats given SPEt (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 6 weeks. Group 7: Diabetic rats given glibenclamide (600 μg/kg body weight) in aqueous solution daily using an intragastric tube for 6 weeks.
All doses were started 48 h after streptozotocin injection. Blood samples were drawn at weekly intervals until the end of study (ie., 6 weeks). At the end of 6th week, all the rats were killed by decapitation (pentobarbitone sodium anaesthesia 60 mg/kg). Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose. Plasma was separated for assay of insulin and other biochemical parameters.

4.7. Analytical methods

Glucose levels were estimated by the O-toluidine method (Sasaki et. al. 1972). Plasma insulin was estimated by using enzyme linked immunosorbant assay (ELISA) kit (Boehringer Mannheim, Mannheim, Germany). Lipid peroxidation in plasma was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) and hydroperoxides by the method of Nichans and Samuelson (1968) and Jiang et al. (1992), respectively. Reduced glutathione (GSH) activity was determined spectrophotometrically by the method of Ellman (1959). Vitamin C and Vitamin E were estimated by the method of Omaye et al. (1979) and Baker et al. (1951). Ceruloplasmin was determined by the method of Ravin (1961).

4.8. Statistical analysis

All data were expressed as mean ± S.D of number of experiments (n = 6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 7.5 (SPSS, Cary, NC, USA) and the individual comparison were obtained by Duncans' Multiple Range Test (DMRT) (Duncan, 1957).

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