Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India.

Effect of an aqueous extract of *Scoparia dulcis* on plasma and tissue glycoproteins in streptozotocin induced diabetic rats

M. Latha, L. Pari

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Dr. L. Pari, Department of Biochemistry, Faculty of Science, Annamalainagar – 608 002, Tamil Nadu, India
paribala@sancharnet.in

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The influence of *Scoparia dulcis*, a traditionally used plant for the treatment of diabetes mellitus, was examined in streptozotocin diabetic rats on dearrangement in glycoprotein levels. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of streptozotocin. An aqueous extract of *Scoparia dulcis* plant was administered orally for 6 weeks. The effect of the *Scoparia dulcis* extract on blood glucose, plasma insulin, plasma and tissue glycoproteins studied was in comparison to glibenclamide. The levels of blood glucose and plasma glycoproteins were increased significantly whereas the level of plasma insulin was significantly decreased in diabetic rats. There was a significant decrease in the level of sialic acid and elevated levels of hexose, hexosamine and fucose in the liver and kidney of streptozotocin diabetic rats. Oral administration of *Scoparia dulcis* plant extract (SPEt) to diabetic rats led to decreased levels of blood glucose and plasma glycoproteins. The levels of plasma insulin and tissue sialic acid were increased whereas the levels of tissue hexose, hexosamine and fucose were near normal. The present study indicates that *Scoparia dulcis* possesses a significant beneficial effect on glycoproteins in addition to its antidiabetic effect.

1. Introduction

Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems. The doubts about the efficacy and safety of oral hypoglycemic agents have prompted a search for safe and effective drugs as alternatives in the treatment of diabetes (Hu et al. 2003). *Scoparia dulcis* L (Scrophulariacae), known as a folk-medicinal plant (common name: Sweet Broomweed), has been used as a remedy for diabetes mellitus in India (Satyanarayana 1969) and for hypertension in Taiwan (Chow et al. 1974). From Indian *Scoparia dulcis*, an antidiabetic compound named amellin was isolated and characterized by Nath (1943). *Scoparia dulcis* is a perennial herb widely distributed in tropical and subtropical regions. The fresh or dried plant has traditionally been used as a remedy for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids and hepatosis and as an analgesic and antipyretic (Farias Freie et al. 1993; Hayashi et al. 1990). A number of compounds including scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol, and scopadulin (Hayashi et al. 1990) have been identified as contributors to the medicinal effects of the plant. These compounds were found to possess various biological activities as inhibitors against replication of herpes simplex virus, gastric H^+, K^+ ATPase activators and antitumor promoters activity etc (Hayashi et al. 1990).

In the diabetic state, glucose is utilized by the insulin independent pathways leading to the synthesis of glycoproteins and even mild deficiency of insulin influences thickening of basement membrane (Konukoglu et al. 1999). Raised levels of glycoproteins in diabetics may also be a predictor of angiopathic complications (Konukoglu et al. 1999).

To our knowledge, no other biochemical investigations had been carried out on the effect of *Scoparia dulcis* in streptozotocin diabetic rats on glycoproteins status and therefore, the present investigation was carried out to study the effect of *Scoparia dulcis* plant extract in streptozotocin diabetic rats on plasma and tissue glycoproteins.

2. Investigations and results

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

The levels of blood glucose, plasma insulin, total haemoglobin and glycosylated haemoglobin of normal and experimental rats are shown in Table 1. There was a significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in streptozotocin diabetic rats when compared with normal rats. Administration of SPEt and glibenclamide tends to bring the parameters significantly towards normal values. The effect of SPEt at a dose of 200 mg/kg body weight was more significant than the effect of 50 and 100 mg/kg body weight and therefore the higher dose was used for further biochemical studies. The administration of SPEt and glibenclamide to normal rats

Table 1: Effect of Scoparia dulcis plant extract (SPEt) on the levels of blood glucose, plasma insulin, haemoglobin and glycosylated haemoglobin in normal and experimental animals

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	83.0 ± 6.8[a]	12.0 ± 1.0[a]	12.00 ± 0.64[a]	0.22 ± 0.02[a]
Normal + SPEt (200 mg/kg)	71.6 ± 4.7[b]	15.1 ± 1.0[b]	13.20 ± 0.57[b]	0.21 ± 0.01[a]
Diabetic control	336.5 ± 13.4[c]	4.1 ± 0.3[c]	8.86 ± 0.08[c]	0.77 ± 0.04[b]
Diabetic + SPEt (50 mg/kg)	108.2 ± 6.8[d]	5.8 ± 0.2[c]	10.41 ± 0.66[d]	0.45 ± 0.02[c]
Diabetic + SPEt (100 mg/kg)	98.5 ± 4.8[e]	8.2 ± 0.6[d]	11.11 ± 0.86[a]	0.42 ± 0.03[c]
Diabetic + SPEt (200 mg/kg)	88.1 ± 6.4[f]	11.0 ± 4.3[e]	11.60 ± 0.96[a]	0.31 ± 0.03[d]
Diabetic + glibenclamide (600 μg/kg)	89.8 ± 6.3[f]	9.8 ± 0.5[f]	10.36 ± 0.92[d]	0.40 ± 0.02[e]

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 2.89, 3.03, 3.13, 3.20, 3.25

showed a significant effect to lower blood glucose and increase plasma insulin while the level of haemoglobin and glycosylated haemoglobin remained unaltered.

2.2. *Effect of SPEt on plasma and tissue glycoproteins*

The levels of plasma and tissue glycoproteins in normal and experimental animals are shown in Table 2. There was a significant increase in the level of plasma glycoproteins in diabetic rats. In liver and kidney of diabetic rats, the levels of hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased. Oral administration of SPEt significantly reversed the changes in plasma, liver and kidney glycoproteins of diabetic rats. The effect of SPEt was better than that of glibenclamide. Normal rats treated with SPEt did not show significant changes.

3. Discussion

In the present investigation, treatment with an aqueous extract of *Scoparia dulcis* showed significant antihyperglycemic activity. The maximum reduction in glucose levels was seen in groups receiving 200 mg/kg of the extract. This is probably indicative of the efficacy of the plant. Moreover, it indirectly indicates that the antihyperglycemic activity of this plant is partly due to a release of insulin from the existing β-cells of the pancreas. The possible mechanism of action of the extract could be correlated with the reminiscent effect of the hypoglycemic sulphonylureas, which promote insulin secretion 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 antihyperglycemic and insulin-release stimulatory effects (Mishkinsky et al. 1974; Venkateswaran and Pari 2002a; Prakasam et al. 2003; Pari and Veukatesvaran 2003; Latha and Pari 2003).

In uncontrolled or poorly controlled diabetes there is increased glycosylation of a number of proteins including haemoglobin (Hb) and α-crystalline of the lens. In long-term diabetes, the glycosylated form of Hb has altered affinity for oxygen and this may be a factor in tissue anoxia (Bunn et al. 1979).

Glycosylated haemoglobin was found to be significantly increased in diabetic animals and the amount of this increase is directly proportional to the fasting blood glucose level (Al-Yassin and Ibrahim 1976). Koenig et al. (1976)

Table 2: Changes in levels of plasma and tissue glycoproteins in normal and experimental animals

Groups	Normal	Normal + SPEt-Aq-200	Diabetic control	Diabetic + SPEt-Aq-200	Diabetic + Glibenclamide
Hexose					
Plasma (mg/dl)	92.6 ± 4.4[a]	90.2 ± 3.8[a]	130.3 ± 6.1[b]	97.6 ± 4.1[c]	106.2 ± 5.4[d]
Liver (mg/g defatted tissue)	28.1 ± 1.3[a]	26.3 ± 1.1[a]	47.2 ± 3.0[b]	32.6 ± 2.4[c]	36.4 ± 2.6[d]
Kidney	22.1 ± 1.0[a]	20.0 ± 1.4[a]	40.6 ± 2.1[b]	28.3 ± 1.8[c]	32.0 ± 2.0[d]
Hexosamine					
Plasma (mg/dl)	75.1 ± 3.6[a]	73.4 ± 2.4[a]	98.2 ± 5.2[b]	80.3 ± 3.1[c]	84.4 ± 4.0[d]
Liver (mg/g defatted tissue)	10.6 ± 0.3[a]	9.1 ± 0.2[a]	18.2 ± 0.8[b]	12.2 ± 0.4[c]	13.0 ± 0.6[c]
Kidney	15.5 ± 0.9[a]	12.2 ± 1.0[a]	28.3 ± 1.9[b]	17.3 ± 1.2[c]	19.2 ± 1.3[c]
Sialic acid					
Plasma (mg/dl)	55.2 ± 2.3[a]	52.1 ± 2.1[b]	67.3 ± 4.3[c]	56.5 ± 3.0[d]	60.3 ± 3.4[e]
Liver (mg/g defatted tissue)	9.2 ± 0.3[a]	9.8 ± 0.2[a]	4.3 ± 0.1[b]	7.0 ± 0.3[c]	6.8 ± 0.2[c]
Kidney	8.6 ± 0.4[a]	9.1 ± 0.6[a]	3.9 ± 0.1[b]	6.2 ± 0.3[c]	5.6 ± 0.2[c]
Fucose					
Plasma (mg/dl)	28.0 ± 1.3[a]	25.2 ± 1.5[b]	42.1 ± 2.3[c]	32.4 ± 1.2[d]	35.0 ± 2.0[e]
Liver (mg/g defatted tissue)	16.2 ± 1.0[a]	15.0 ± 1.3[a]	28.2 ± 2.2[b]	18.4 ± 1.1[c]	20.1 ± 1.5[c]
Kidney	13.0 ± 0.9[a]	12.2 ± 1.0[a]	26.3 ± 1.5[b]	15.8 ± 1.1[c]	18.4 ± 1.2[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)
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also reported a 16% increase in the level of glycosylated haemoglobin in diabetic patients. The level of total haemoglobin was found to be decreased in the diabetic group and this may be due to the increased formation of glycosylated haemoglobin. This was well correlated with earlier studies, which reported that there was a decrease in the level of haemoglobin in experimental diabetic rats (Venkateswaran and Pari 2002b). The increase in the level of haemoglobin in animals given SPEt may be due to the decreased level of blood glucose.

Generalized abnormalities in glycoproteins metabolism is observed in both naturally occurring and experimental diabetes (Mc Millan 1970). Berenson et al. (1972) reported that streptozotocin diabetic rats exhibited a significant modification in the connective tissue macromolecule. Insulin has been shown to increase the incorporation of glucose in the rat submaxillary gland (Konukoglu et al. 1999). The requirement of insulin for the biosynthesis of the carbohydrate moiety of mucoproteins from glucose is thus evident. A decreased incorporation in diabetic rats may be due to insulin deficiency.

The increases in plasma glycoprotein components have been reported to be associated with severity and duration of diabetes. Previous reports suggest that serum concentrations of glycoproteins are significantly increased in diabetes mellitus (Mc Millan 1970). Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in plasma (Radhakrishnamoorthy and Berenson 1973). Therefore, vascular complications that involve complex protein-carbohydrate molecules could contribute to an increase in plasma glycoproteins.

The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin independent pathway for the utilization of glucose-6-phosphate. But the deficiency of insulin during diabetes produces derrangement of glycoprotein metabolism, resulting in the thickening of the basal membrane. The increased availability of glucose in the hyperglycemic state accelerates the synthesis of basement membrane components i.e., glycoproteins (Spiro and Spiro 1971). This is due to a depressed utilization of glucose by insulin dependent pathways, thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins (Patti et al. 1999).

The decrease in the content of sialic acid in tissues may be due to the utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure (Schiller and Dorfman 1957). The synthesis of fibronectin was also reported to increase significantly in various tissues of diabetic patients and animals (Schiller and Dorfman 1957). Administration of SPEt to diabetic rats significantly reversed all these changes to near normal levels.

The antihyperglycemic action of SPEt, which is mediated via an enhancement of insulin action, as it is evident by the increased level of insulin in diabetic rats treated with SPEt, may be responsible for the reversal of glycoprotein changes.

In conclusion, the administration of SPEt to diabetic rats has a beneficial effect on the carbohydrate moieties of glycoproteins.

3. Experimental

3.1. 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 with 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.

3.2. 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.

3.3. 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 the Botany Directorate in Annamalai University. A voucher specimen (No. 3412) was deposited in the Botany Department of Annamalai University.

3.4. Preparation of plant extract

Scoparia dulcis fresh whole plants (500 g) 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).

3.5. 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 (Siddiqui et al. 1987). 48 h after streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e with blood glucose of 200–300 mg/dl) were taken for the experiment.

3.6. Experimental design

In the experiment, 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 suspension daily using an intragastric tube for 6 weeks.

All doses were started after 48 h 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 a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose and the plasma was separated for the estimation of insulin and glycoproteins. Liver and kidney were dissected out, washed in ice cold saline, patted dry and weighed.

3.7. Analytical methods

Glucose levels were estimated by the O-toluidine method (Sasaki et.al. 1972). Plasma insulin was estimated by an enzyme linked immunosorbant assay (ELISA) kit (Boehringer Mannheim, Mannheim, Germany), using rat insulin as standard. Haemoglobin was estimated by the method of Drabkin and Austin (1932). Glycosylated haemoglobin (HbA_1C) was estimated by the method of Sudhakar Nayak and Pattabiraman (1982) with modifications according to Bannon (1982). For the estimation of glycoproteins, the tissues were defatted by the method of Folch et al. (1957) and the defatted tissues were treated with 0.1N H_2SO_4 and hydrolysed at 80 °C and aliquots were used for sialic acid estimation. To the remaining solution, 0.1 N NaOH was added. The aliquots were used for fucose, hexose, and hexosamine estimation. Hexose was estimated by the method of Niebes (1972). The reaction mixture contained 0.5 ml of aliquot/plasma, 0.5 ml of 5% phenol and 2.5 ml of conc. H_2SO_4 and boiled for 20 min and the absorbance was read at 490 nm. Hexosamine was determined by the method of Elson and Morgan (1933) with slight modifications of Niebes (1972). Briefly, the reaction mixture containing 0.5 ml plasma/1.0 ml aliquot, 2.5 ml of 3N HCl was boiled over 6 h and neutralized with 6 N NaOH. To 0.8 ml of neutralized sample 0.6 ml of acetyl acetone reagent was added and boiled for 30 min. The mixture was treated with 2.0 ml of Ehrlich's reagent. The colour developed was read at 540 nm colorimetrically.

Sialic acid and Fucose were determined by the method of Warren (1959) and Deist and Shuttle (1948). In brief, 0.5 ml of aliquot/plasma, was treated with 0.5 ml of water, 0.25 ml of periodic acid and incubated at 37 °C for 30 min. To the reaction mixture 0.2 ml of sodium meta arsenate and

2.0 ml of thiobarbituric acid were added, heated for 6 min and 5.0 ml of acidified butanol were added. The absorbance was read at 540 nm.
For fucose estimation, 0.5 ml of aliquot/plasma were treated with 4.5 ml of H_2SO_4 and boiled for 3 min. 0.1 ml of cysteine hydrochloride reagent was added. After 75 min in the dark, the absorbance was read at 393 and 430 nm.

3.8. 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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