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ORIGINAL RESEARCH ARTICLE

Antidiabetic Effect of Fraxetin: Protective Role on the Levels of Glycoprotein Components in Experimental Diabetic Rats

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ABSTRACT

The present study was conducted to investigate the effect of fraxetin on dearrangement in glycoprotein levels in the streptozotocin (STZ)-induced diabetic model. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (40 mg/kg b.w). The levels of glycoproteins were altered in experimental diabetes mellitus. Fraxetin (80 mg/kg b.w) was administered orally for 30 days. The effects of fraxetin on plasma glucose, insulin, plasma and tissue glycoproteins were studied. Oral administration of fraxetin (80 mg/kg b.w) for 30 days positively modulates the glycemic status in STZ-induced diabetic rats. The levels of plasma glucose were decreased with significant increase of plasma insulin level. The altered levels of plasma and tissue glycoprotein components were restored to near normal. The present findings suggest that fraxetin can potentially ameliorate glycoprotein components abnormalities in addition to its antihyperglycemic effect in experimental diabetes. In light of these advantageous results, it is advisable to broaden the scale of use of fraxetin in a trial to alleviate the adverse effects of diabetes.

Key words: Fraxetin, Diabetes mellitus, Glycoproteins, Insulin, Streptozotocin

INTRODUCTION

Diabetes mellitus, a life threatening as well as life style modifying metabolic disorder, is manifested mainly by hyperglycemia, which is due to defect in insulin secretion, function and or both [1]. Hyperglycemia leads to several acute and long-term complications if it persists for longer time. Hyperglycemia in experimental diabetic rats leads to a decreased utilization of glucose by insulin dependent pathways, thereby enhancing the formation of glycoproteins and increased polyol and hexosamine pathway. Diabetes is showing an alarming increase in prevalence, especially in developing countries such as India [2]. India now has more than 50 million people with type 2 diabetes [2], which is characterized by fasting and postprandial hyperglycemia and relative insulin insufficiency. The vast majority of diabetes will be of type 2 diabetes mellitus. Type 2 diabetes now affects 5.9% of the world's adult population with almost 80% of the total in developing countries [3]. There are about 200 million people around the world who are suffering from type 2 diabetes mellitus and the number is expected to reach 300 million cases by the year 2025 [4].

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which form the principle components of animal cells. Hexoses, hexosamine and sialic acid are the basic components of the glycoproteins. They play an important role in membrane transport, cell differentiation and recognition, the adhesion of macromolecules to cell surface and the secretion and absorption of macromolecules [5]. Several workers have suggested that elevated levels of glycoproteins in plasma, liver and kidney tissues in the diabetic condition could be a consequence of impaired carbohydrate metabolism. Insulin deficiency and high levels of plasma glucose in the diabetic condition may result in an increased synthesis of glycoproteins. This increase in plasma glycoproteins has been associated with the severity and duration of diabetes.

Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, has been widely used for inducing diabetes in the experimental

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animals through its toxic effects on pancreatic β-cells [6]. Currently available drugs for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure. As a complementary/alternative approach, plant bioactive constituents with anti-hyperglycemic activities are increasingly sought after by diabetic patients and healthcare professionals [7]. Coumarins are secondary metabolites widely distributed in the plant kingdom. Modern pharmaceutical studies have proved that coumarins and their derivatives have a wide range of bioactivities [8]. They are also found in natural food products, such as citrus fruits, tomatoes, vegetables and green tea. Fraxetin (7,8-dihydroxy-6-methoxy coumarin), a coumarin derivative, has been reported to possess antioxidative, anti-inflammatory, antiviral, antitumor and neuroprotective effects [9,10].

The present study is aimed to investigate ameliorative potential of fraxetin on glucose, insulin and glycoprotein components (hexose, hexosamine, fucose and sialic acid) in plasma and tissues (liver and kidney) of STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals:

Male Wistar rats weighing 150 to 200 g used for the study were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. (4 rats per cage) at an ambient temperature of 25°C with 12-hour light to 12-hour dark cycle. Rats had free access to standard food and water *ad libitum*. The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of the experiment. All experimental procedures were conducted according to the institutional animal ethical committee (Reg No.856/2012) and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Chemicals:

Fraxetin and STZ were purchased from Sigma Chemical Co (St. Louis, Mo. USA). All other chemicals and solvents were of analytical grade and purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

Induction of diabetes:

Diabetes was induced in overnight fasted experimental rats by a single intraperitoneal injection of STZ (40 mg/kg b.w) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). STZ injected animals were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. Control rats were injected with same volume of citrate buffer alone. After 96 h, plasma glucose was determined and those rats with fasting blood glucose greater than 250 mg/dl were used in the present study.

Experimental design

The animals were randomly divided into four groups of six animals in each group (24 diabetic surviving and 12 normal). Fraxetin was dissolved in vehicle solution of 1.0% dimethylsulfoxide (DMSO) and administered to experimental rats.

Group I: Normal control (vehicle treated)
Group II: Normal rats received fraxetin (80 mg/kg b.w) dissolved in 1 ml of 1.0% DMSO intra gastrically for 30 days
Group III: Diabetic control
Group IV: Diabetic rats received fraxetin (80 mg/kg b.w) dissolved in 1 ml of 1.0% DMSO intra gastrically for 30 days

After 30 days of treatment, the animals were deprived of food overnight, anaesthetized and sacrificed by cervical decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of glucose, insulin and glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

Extraction of glycoproteins:

To 0.1 ml of plasma, 5.0 ml of methanol was added, mixed well and centrifuged for 10 min at 3000×g. The supernatant was decanted and the precipitate was again washed with 5.0 ml of 95% ethanol, recentrifuged and the supernatant was decanted to obtain the precipitate of glycoproteins. This was used for the estimation of hexose and hexosamine. For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 ml of methanol. The contents were filtered and homogenized with 14.0 ml of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1v/v) and each time the extract was filtered. The residue (defatted tissues) was obtained and the filtrate decanted. A weighed amount of defatted tissue was suspended in 3.0 ml of 2 N HCl and heated at 90°C for 4 h. The sample was cooled and neutralized with 3.0 ml of 2 N NaOH. Aliquots from this were used for

estimation of fucose, hexose, hexosamine and sialic acid.

BIOCHEMICAL ASSAYS

Determination of plasma glucose and insulin:

The level of plasma glucose was estimated spectrophotometrically according to the method of Trinder [11], using commercial diagnostic kit (Randox Laboratories, UK). Plasma insulin was assayed by ELISA using a Boehringer–Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Both the analyses were done according to the manufacturer's instructions.

Determination of glycoproteins:

The plasma and tissue hexose content was estimated by the method of Niebes [12], sialic acid in plasma and tissues were estimated by the method of Warren [13] and hexosamine by the method of Wagner [14]. Fucose was estimated by the method of Dische and Shettles [15], respectively.

Statistical analysis

Data presented as means ± SD and subjected to statistical significance were evaluated by one way analysis of variance (ANOVA) using SPSS Version 16.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan's Multiple Range Test (DMRT). Values are considered statistically significant when $p<0.05$[16].

RESULTS

Effect of fraxetin on the levels of plasma glucose and insulin

(**Table 1**) shows the level of plasma glucose and insulin in control and experimental diabetic animals. There was a significant elevation in plasma glucose level with significant decrease in plasma insulin levels in STZ-induced diabetic rats, compared with normal rats. Administration of fraxetin tended to bring plasma glucose and insulin towards near normal levels. The plasma glucose and insulin levels of normal rats were not altered when administered with fraxetin (80 mg/kg b.w).

Effect of fraxetin on the levels of plasma glycoproteins

(**Table 2**) shows shows the changes in the levels protein bound hexose, hexosamine, fucose and sialic acid in plasma of control and experimental rats. Significantly higher levels of glycoprotein components were observed in the plasma of diabetic rats when compared to normal control rats. Administration of fraxetin to diabetic rats resulted in a significant reduction of protein bound hexose, hexosamine, fucose and sialic acid in plasma when compared to diabetic control rats.

Effect of fraxetin on the levels of tissue glycoproteins

The levels of liver and kidney glycoprotein of control and experimental rats were shown in (**Tables 3 & 4**). The level of hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased and those levels were brought back to near normal by treatment with fraxetin.

Table 1: Effect of fraxetin on the levels of plasma glucose and insulin in control and experimental rats

Groups	Plasma glucose (mg/dL)	Plasma insulin (µu/dL)
Normal control	75.97 ± 5.35[a]	15.84 ± 0.24[a]
Normal + fraxetin (80mg/kg)	76.34 ± 5.58[a]	14.32 ± 0.22[a]
Diabetic control	299.35 ± 23.59[b]	5.44 ± 0.12[b]
Diabetic + fraxetin (80mg/kg)	96.74 ± 6.23[c]	12.04 ± 0.18[c]

Values are given as means ± S.D. for six rats in each group.
Values in a column not sharing a common superscript symbol [(a -c)] differ significantly at $p<0.05$. Duncan's Multiple Range Test (DMRT).

Table 2: Effect of fraxetin on plasma glycoprotein levels in normal and experimental rats

Groups	Hexose	Hexosamine	Fucose	Sialic acid
	(mg/dl)			
Normal	94.30 ± 7.02[a]	75.26 ± 6.21[a]	31.28 ± 2.62[a]	52.74 ± 3.71[a]
Normal +fraxetin (80mg/kg)	89.43 ± 6.85[a]	72.51 ± 5.71[a]	29.13 ± 2.02[a]	50.22 ± 4.01[a]
Diabetic control	157.21 ± 12.32[b]	96.35 ± 7.24[b]	48.54 ± 4.55[b]	77.53 ± 5.87[b]
Diabetic+fraxetin (80mg/kg)	107.28 ± 8.12[c]	80.37 ± 6.12[c]	36.63 ± 2.86[c]	59.12 ± 4.02[c]

Values are given as means ± S.D. for six rats in each group.
Values in a column not sharing a common superscript symbol [(a -c)] differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 3: Effect of fraxetin on liver glycoprotein levels in normal and experimental rats

Groups	Hexose	Hexosamine	Fucose	Sialic acid
	(mg/g defatted tissue)			
Normal	28.32 ± 2.09[a]	12.65 ± 0.95[a]	18.21 ± 1.73[a]	10.07 ± 0.85[a]
Normal + fraxetin (80mg/kg)	26.74 ± 1.99[a]	11.23 ± 0.88[a]	17.20 ± 1.3[a]	10.95 ± 0.88[a]
Diabetic control	49.64 ± 4.22[b]	22.75 ± 2.03[b]	31.01 ± 3.02[b]	4.69 ± 0.37[b]
Diabetic + fraxetin (80mg/kg)	34.31 ± 2.63[c]	16.93 ± 1.32[c]	22.18 ± 1.63[c]	7.94 ± 0.71[c]

Values are given as means ± S.D. for six rats in each group.
Values in a column not sharing a common superscript symbol [(a -c)] differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 4: Effect of fraxetin on kidney glycoprotein levels in normal and experimental rats

Groups	Hexose	Hexosamine	Fucose	Sialic acid
	(mg/g defatted tissue)			
Normal	27.36 ± 2.54[a]	17.55 ± 1.74[a]	15.28 ± 1.47[a]	9.79 ± 0.89[a]
Normal + fraxetin (80mg/kg)	25.29 ± 2.02[a]	15.91 ± 1.46[a]	14.61 ± 1.10[a]	10.08 ± 0.81[a]
Diabetic control	44.26 ± 3.92[b]	35.52 ± 2.82[b]	33.73 ± 2.93[b]	6.19 ± 0.60[b]
Diabetic + fraxetin (80mg/kg)	32.72 ± 3.54[c]	23.29 ± 2.05[c]	19.45 ± 1.81[c]	7.61 ± 0.64[c]

Values are given as means ± S.D. for six rats in each group.
Values in a column not sharing a common superscript symbol [(a -c)] differ significantly at $p<0.05$. Duncan's Multiple Range Test (DMRT).

DISCUSSION

Streptozotocin-induced hyperglycemia in animals is considered to be a good model for the preliminary screening of agents active against diabetes. The mechanism by which STZ brings about its diabetic state includes selective destruction of pancreatic β-cells which make cells less active [17], leading to poor sensitivity of insulin for glucose uptake by tissues, causes hyperglycemia. From the results obtained, it is evident that diabetic rats had much higher glucose level than of control rats. Low dose of STZ (40 mg/kg body weight) destroy some population of pancreatic β-cells in rats leading to insufficient insulin secretion causing type 2 diabetic model Persistent hyperglycemia, the common characteristic of diabetes can cause most diabetic complications and it is normalized by the action of insulin [18]. Oral administration of fraxetin decreased the blood glucose level in diabetic rats. The possible mechanism by which fraxetin brings about decrease in blood glucose in STZ-induced diabetes may be by preventing the death of β-cells and/or it may permit the recovery of partially destroyed β-cells [19]. It is possible that fraxetin may have initiated cell proliferation, since it has been reported that pancreatic endocrine cells have the potential to proliferate after induction of diabetes with STZ [20]. Our results are in harmony with Prabakaran and Ashokkumar [21] who reported that administration of esculetin, a coumarin compound to diabetic rats significantly decreased the glucose level to near normal through enhanced release of insulin from the existing β-cells.

Glycation is a nonenzymatic reaction of glucose and other saccharide derivatives with proteins, nucleotides and lipids. Glycation occurs inside and outside of cells; Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. These effects are countered by protein degradation and renewal. Glycation of the extracellular matrix produces changes in macromolecular structure affecting cell–cell and cell–matrix interactions associated with decreased elasticity and increased fluid filtration across arterial wall and endothelial cell adhesion [22]. In this study, elevated levels of glycoproteins are observed in plasma, liver and kidney in diabetic rats. Increased glycosylation of various proteins in diabetic patients had been reported earlier [23].

Liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus. In experimental diabetes, STZ exerts its toxic effect on liver and other organs in addition to pancreatic β-cells. The kidney reabsorbs 99% of plasma glucose that filters through tubules in normal individuals via mechanisms independent of insulin. It is estimated that the filter load of glucose is 180 mg/ day, and only 500 mg of glucose is excreted in urine during the day. In the diabetic state, a deficiency in insulin secretion causes derangement of glycoprotein metabolism, which results in basal membrane thickening. Excess availability of glucose in the hyperglycemic state accelerates the synthesis of glucose basement membrane components i.e. glycoproteins [24]. The biochemical markers, hexose, hexosamine, fucose and sialic acid have been measured in the liver and kidney because liver is responsible for synthesis of all major proteins, which are then secreted into the blood.

Hexosamines are amino sugars created by adding an amine group to a hexose. The level of hexosamine, increased significantly in the plasma, liver and kidney of diabetic rats, which may be due to insulin deficiency. In diabetic rats treated with fraxetin significantly lowered hexosamine, which might be due to increased secretion of insulin.

Fucose is a member of the group of eight essential sugars the body requires for optimal function of cell-to-cell communication and its metabolism appears to be altered in various diseases such as diabetes mellitus [25]. A raise in fucose levels could be due to increased glycosylation in the diabetic state. Elevated levels of fucose in

experimental diabetes were reported by other researchers [26]. In diabetic rats treated with fraxetin significantly lowered fucose levels, which might be due to increased secretion of insulin. Our results are finding in line with the study of reduced fucose by improved secretion of insulin in coumarin treated diabetic rats [27].

An elevation in plasma sialic acid levels is observed in type 2 diabetes mellitus and is also a risk factor for micro vascular complications [28]. Oxidative stress and inflammation brings damages to cellular membranes and increases plasma sialic acid levels. In addition, vascular endothelium is rich in sialic acid moieties where it regulates permeability. Impaired function of insulin and the resulting hyperglycemia are associated with endothelial dysfunction leading to the release of sialic acid into circulation [29]. Thus in diabetes there is a consistent increase in plasma sialic acid levels whereas its content varies in different tissues [30]. The diminished activity of enzymes of sialic acid biosynthesis explains the decreased sialic acid content in diabetic liver of experimental animals. This decrease may also be related to increased synthesis of fibronectin, which contains sialic acid in its core structure. Treatment with fraxetin had restored sialic acid level to near normal, which could be due to improved glycemic status in plasma and tissues. These results are agreed with pari and srinivasan [31] who reported that diosmin, a citrus flavonoid improves sialic acid level in diabetic rats.

From the above findings, we conclude that oral administration of fraxetin possesses glucose lowering effect in STZ-induced diabetic rats. It also improved plasma insulin levels and decreased glycoprotein components in plasma, liver and kidney. This can be used as an effective indicator to show the beneficial effects of fraxetin in controlling the progression and complications of diabetes.

CONFLICT OF INTEREST

The authors of this article do not have any conflict of interest to disclose. No part of the manuscript has been submitted or is under consideration in any other publication.

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