



Protective Role of Chrysoeriol, A Bioactive Flavonoid on Plasma and Tissue Glycoprotein Component Level Changes in Streptozotocin-Induced Hyperglycemic Rats

*Baskaran. K, Nirmaladevi. N, Sumi.P and Jyothylakshmy Rajan
Department of Biochemistry, Sree Narayana Guru College, Coimbatore, Tamilnadu, India.

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*Corresponding Author Email: baskar.bio86@gmail.com

Abstract

The present study was conducted to investigate the effect of Chrysoeriol (CS) on dearrangement in glycoprotein levels in the streptozotocin (STZ) -induced diabetic model. Diabetes was induced in Male Wistar rats by a single intraperitoneal (i.p) injection of STZ (40 mg/kg B.W). The levels of glycoproteins were altered in experimental diabetes mellitus. The effects of CS on plasma glucose, insulin, plasma and tissue glycoproteins were studied. Oral administration of CS (20 mg/kg b.w) for 45 days positively modulates the glycemic status in STZ-induced diabetic rats. The levels of plasma glucose were significantly decreased and increased of plasma insulin level. The altered levels of plasma and tissue glycoprotein components were restored to near normal. The present findings suggest that CS 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 CS in a trial to alleviate the adverse effects of diabetes.

Keywords

Chrysoeriol, Diabetes mellitus, Glycoproteins, Insulin, Streptozotocin

INTRODUCTION

Diabetes mellitus, a life threatening as well as lifestyle modifying metabolic disorder, is manifested mainly by hyperglycemia, which is due to defect in insulin secretion, function and or both (1). It is a serious and progressive disorder affecting approximately 60% of the Asian population and according to World Health Organization, the number of diabetic patients is expected to increase 366 million or more by the year 2030 (2,3). Hyperglycemia and insulin deficiency, the hallmarks of diabetes mellitus alter glycoprotein components in various tissues. Several studies reported that impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetic complications.

Glycoproteins are conjugated proteins that contain one or more covalently linked carbohydrate chains which contribute to the structure of the extracellular matrix in animal cells (4). Hexose, hexosamine and sialic acid are the basic components of cell surface glycoproteins, which play important roles in cell differentiation and recognition, adhesion of macromolecules to the cell surface and in the secretion and absorption of macromolecules (5). They also serve numerous biological functions like blood group antigens, enzymes, and transporters. Derangement in the metabolism of hexose, hexosamine, fucose and sialic acid has been observed in naturally occurring and in experimental diabetes. Various studies have suggested that

alteration in glycoprotein components could be a consequence of impaired carbohydrate metabolism (6,7).

In our laboratory was highly encouraging and revealed a significant blood glucose lowering effect after oral administration of CH leaf extract in normal and STZ induced diabetic rats and no harmful side effects were observed throughout the study (8,9). Chrysoeriol (5, 7 – dihydroxy – 2 - (4 – hydroxy - 3-methoxyphenyl) chromen-4-one) and diosmetin were only possessed many biological effects similar to luteolin, such as antioxidant, anti-inflammatory, and anti-osteoporotic effects (10,11). Furthermore, CS and diosmetin were natural pro drugs in cancer prevention (12,13). The structure of CS is depicted below (Fig.1).

The present study is aimed to investigate the ameliorative potential of CS 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

Animals Adult Male albino Wistar rats (9 weeks old; 180–200 g) were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned

Group I	: Control (0.5% DMSO)
Group II	: Control + Chrysoeriol (20 mg/kg BW)
Group III	: Diabetic control (0.5% DMSO)
Group IV	: Diabetic + Chrysoeriol (20 mg/kg BW)
Group V	: Diabetic + glibenclamide (600 µg/kg BW)

After 45 days of treatment, the animals were deprived of food overnight, anaesthetized and sacrificed by cervical decapitation. The blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma 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

room (25 ± 1°C) with a 12 h light/dark cycle. Feed and water were provided ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalai University (Reg. No. 160/1999/CPCSEA, Proposal No:539).

Chemicals:

STZ was 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:

DM was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of STZ (40 mg/kg BW) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). STZ injected rats were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemia mortality. The induction of DM in rats was confirmed by estimating the elevated plasma glucose levels, 72 h after STZ injection. Rats with fasting plasma glucose levels more than 250 mg/dl were considered diabetic and chosen for the study.

Experimental design (45 days)

The animals were randomly divided into five groups of six animals each. CS and glibenclamide were administered post-orally by intubation once in a day in the morning for 45 days.

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 (14) 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, sialic acid, hexosamine and Fucose content was estimated by the method (15-18) respectively.

Statistical analysis

Data presented as means \pm 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 $p < 0.05$.

RESULTS

Effect of CS 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 a significant decrease in plasma insulin levels in STZ-induced diabetic rats, compared with normal rats. Administration of CS 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 CS (20 mg/kg b.w).

Effect of CS on the levels of plasma glycoproteins (Table 2) 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 CS 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 CS 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 CS.

Table 1. Effect of CS on fasting plasma glucose and insulin in STZ -induced diabetic rat

Groups	Plasma glucose (mg/dL)	Insulin (μ U/mL)
Control	98.56 \pm 6.72	16.27 \pm 1.36
Control + CS (20 mg/kg BW)	95.22 \pm 7.94	17.56 \pm 1.22
Diabetic control	270.46 \pm 20.41	7.03 \pm 0.48
Diabetic + CS (20 mg/kg BW)	119.02 \pm 8.56	14.54 \pm 1.12
Diabetic + Glibenclamide (600 μ g/kg BW)	110.76 \pm 9.23	15.38 \pm 1.31

Values are given as means \pm S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

Table 2. Effect of CS on plasma glycoproteins in normal and STZ-induced diabetic rats

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)	Sialic acid (mg/dl)
Control	92.46 \pm 6.82 ^a	73.62 \pm 5.09 ^a	29.86 \pm 1.99 ^a	48.47 \pm 3.29 ^a
Control + CS (20 mg/kg BW)	90.22 \pm 6.45 ^a	70.19 \pm 5.76 ^a	27.10 \pm 1.76 ^a	46.31 \pm 3.87 ^a
Diabetic control	150.37 \pm 11.77 ^b	95.31 \pm 6.89 ^b	45.46 \pm 3.82 ^b	76.73 \pm 6.20 ^b
Diabetic + CS (20 mg/kg BW)	105.82 \pm 7.66 ^c	82.75 \pm 6.02 ^c	34.36 \pm 2.74 ^c	55.14 \pm 4.64 ^c
Diabetic + Glibenclamide (600 μ g/kg BW)	101.12 \pm 7.92 ^c	80.84 \pm 5.27 ^c	31.72 \pm 2.36 ^c	51.45 \pm 3.37 ^d

Values are given as means \pm S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

Table 3. Effect of CS on liver glycoproteins in normal and STZ -induced diabetic rats

Groups	Hexose (mg/100 g tissue)	Hexosamine	Fucose	Sialic acid
Control	25.72 \pm 1.78 ^a	13.52 \pm 1.07 ^a	15.38 \pm 0.99 ^a	12.02 \pm 0.89 ^a
Control + CS (20 mg/kg BW)	23.09 \pm 1.54 ^a	11.36 \pm 0.98 ^a	14.42 \pm 1.11 ^a	11.90 \pm 0.72 ^a
Diabetic control	45.47 \pm 3.02 ^b	24.54 \pm 2.01 ^b	33.08 \pm 2.82 ^b	4.02 \pm 0.27 ^b
Diabetic + CS (20 mg/kg BW)	32.19 \pm 2.26 ^c	18.93 \pm 1.46 ^c	24.16 \pm 1.57 ^c	8.49 \pm 0.67 ^c
Diabetic + Glibenclamide (600 μ g/kg BW)	30.41 \pm 2.12 ^c	16.27 \pm 1.24 ^c	20.23 \pm 1.32 ^c	7.26 \pm 0.53 ^c

Values are given as means \pm S.D. for six rats in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

Table 4. Effect of CS on kidney glycoproteins in normal and STZ -induced diabetic rats

Groups	Hexose (mg/100 g tissue)	Hexosamine	Fucose	Sialic acid
Control	29.52±2.42 ^a	18.27±1.36 ^a	14.17±1.02 ^a	10.01±0.80 ^a
Control + CS (20 mg/kg BW)	27.08±1.94 ^a	15.86±1.12 ^a	12.46±1.35 ^a	9.27±0.72 ^a
Diabetic control	42.86±3.45 ^b	37.25±2.76 ^b	32.24±2.79 ^b	5.12±0.47 ^b
Diabetic + CS (20 mg/kg BW)	34.22±3.21 ^c	26.91±2.01 ^c	19.56±1.76 ^c	8.37±0.63 ^c
Diabetic + Glibenclamide (600 µg/kg BW)	32.17±2.04 ^c	23.46±1.75 ^c	17.43±1.25 ^c	7.16±0.51 ^c

Values are given as means ± S.D. for six rats in each group.
 Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

DISCUSSION

Streptozotocin selectively destroys the pancreatic insulin secreting β -cells, leaving fewer active cells and resulting in a diabetic state. It reliably produces many of the signs and symptoms of chronic human diabetes (19). Streptozotocin is a potent DNA methylating agent and acts as a nitric oxide donor in pancreatic cells. Beta cells are particularly susceptible to damage by streptozotocin, a cytotoxic agent (20). As a result, the expression and secretion of insulin declines, notably, thereby leading to hyperglycemia, a clinical hallmark of diabetes. From the results obtained, it is evident that diabetic rats had much higher glucose levels than control rats. Some substances have shown antidiabetic effects by influencing beta cells to stimulate insulin secretion and restore insulin sensitivity (21,22). Oral administration of CS resulted in a significant reduction in blood glucose. Flavonoids are one of the most numerous and widespread groups of phenolic compounds in higher plants. That decrease may be due to the insulin release effect of diosgenin on peripheral tissues, either by promoting glucose uptake and metabolism or by inhibiting hepatic gluconeogenesis (23).

Glycoproteins together with glycosaminoglycans form the major macromolecular components of connective tissue (24). The luminal surface of epithelial cells in kidney tubules is also lined with a thick carbohydrate rich glycoprotein layer (25). Diabetes mellitus is reflected in profound changes in the metabolism of glycoproteins (26,27) and by a reduction in membrane glycosylation in the kidney brush border membrane (28).

The structural and functional alterations of both circulating and membrane bound proteins is the result of prolonged elevation of blood glucose and insulin deficiency in diabetes and also the deficiency of insulin results in the thickening of the basal membrane of pancreatic beta cells (29). The alterations in the composition of carbohydrate components of glycoproteins of serum and basement membrane have been reported in the condition like diabetes (30). The increased plasma glycoprotein

components have been associated with the severity of diabetes. The elevation in the levels of plasma glycoprotein components might be due to the secretion from cell membrane glycoconjugates in the circulation (31). In the present study, we observed the increased levels of hexose, hexoseamine, fucose and salic acid in the plasma of STZ induced diabetic rats. Administration of CS ameliorates the levels of plasma glycoproteins near normal. Our results are in agreement with Basha and Sankaranarayanan., who reported that β -caryophyllene improved glycoprotein levels in diabetic rats (32).

The liver plays pivotal role in producing a large amount of glycoproteins present in blood. The elevated levels of plasma glycoproteins in diabetic condition could be a consequence of abnormal carbohydrate metabolism (33,34). Numerous molecular mechanisms are concerned with hyperglycemia induced metabolic disturbances in diabetes. Among these hexosamine biosynthetic pathway represents a minor metabolic route of glucose at fructose 6- phosphate step of glycolysis. This pathway is considered as a sensor of nutrients and an increase in this pathway is regarded as a key factor in the metabolic complications of diabetes (35). Prolonged hyperglycemia due to insulin deficiency associated with oxidative stress increases the expression of GFAT (Glutamine: Fructose 6- phosphate amino transferase), the rate-limiting enzyme of this pathway leading to an increase in the levels of hexosamine (36). Hexosamines function as physiologic glucose sensors that serve as an adaptor in redirecting excess calories just before storage as fat (37). The results of the present study are in harmony with previous studies that diabetic rats showed elevated levels of hexosamines, which could be due to, increased expression of GFA and increased plasma glucose. Our study indicates that the elevated levels of hexosamine were observed in plasma and tissues of diabetic rats when compared with normal control rats. Diabetic rats treated with CS and glibenclamide showed significantly decreased hexosamines in the plasma and tissues when

compared to diabetic rats, which could be due to improved glycemic control.

L-fucose, a deoxyhexose is a component of many N- and O-linked glycoproteins and participates in many biological recognition events. Fucose and sialic acid form specific, structures called glycanic chains covalently linked to lipids or proteins which are present on the cell surface (7). Fucosylated glycans are synthesized from fucosyl transfers and have important roles in selecting-mediated leukocyte-endothelial adhesion and in blood transfusion reactions (38). In diabetic state, the levels of fucose is significantly increased which may be due to the increased activities of fucosidase and fucosyl transferase (39). In diabetic rats treated with CS 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 fraxetin, TA & carvone treated diabetic rats (40-42).

Diabetes leads to the progression of microvascular pathology in renal glomerulus and the end-stage of renal disease is the consequence of its microvascular pathology (35). The hyperglycemia mediated oxidative stress and inflammation may contribute to bringing about damages to cellular membranes and increases serum salicylic acid levels. In addition to this, vascular endothelium is rich in salicylic acid moieties where it regulates permeability. Impaired function of insulin and the resulting hyperglycemia are associated with impairment in endothelial, leading to the release of salicylic acid into circulation (42). The diminished content of salicylic acid in the tissues might be due to the utilization for the synthesis of fibronectin, which have salicylic acid residues in the core structure. The epithelial cells of the luminal surface in kidney tubules are also lined with a thick carbohydrate rich glycoprotein layer (24). The administration of CS increased the content of salicylic acid in the tissues and decreased the levels of salicylic acid levels in the plasma. This decrease may also be related to increased synthesis of fibronectin, which contains sialic acid in its core structure. This is attributed to the insulinotropic potential of CS which restored the altered glycoprotein components in the plasma and tissues of diabetic animals to near normal. Our results are in accordance with the previous reports (34,40&44).

CONCLUSIONS

In conclusion, we put forward that the intragastric administration of CS (20 mg/kg BW) to the diabetic rats resulted in modulation of glycoprotein content in plasma, liver and kidney. Supplementation of CS was shown to be effective in diabetic complications

by the enhancement of insulin action, as evidenced by the decreased level of plasma glucose in diabetic rats treated with CS. In addition, our findings provided an insight that CS could be developed as a new food additive or a drug ingredient for the prevention of diabetes mellitus.

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