Int J Biol Med Res. 2010; 1(3): 61-65.

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International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original article

Protective role of coumarin on plasma and tissue glycoprotein components in streptozotocin- nicotinamide induced hyperglycemic rats

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ARTICLE INFO

Keywords:
Coumarin
Diabetes
Glycoproteins
Streptozotocin

ABSTRACT

The present investigation was carried out to evaluate the protective role of coumarin on glycoprotein metabolism in streptozotocin (STZ)-nicotinamide (NA) induced type 2 diabetic rats. Coumarin was administered orally (100 mg/kg bodyweight) for 45 days to normal and diabetic rats. The effects of coumarin on plasma and tissue glycoproteins (hexose, hexosamine, fucose and sialic acid) were determined. The levels of plasma glycoproteins containing hexose, hexosamine, sialic acid and fucose were significantly increased in diabetic rats when compared with normal control 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 STZ-NA induced diabetic rats. On oral administration of coumarin to diabetic rats showed decreased levels of plasma glycoproteins. The level of tissue sialic acid was increased whereas the levels of tissue hexose, hexosamine and fucose were reversed to near normal. The present study indicates that the coumarin possesses a significant protective effect on glycoprotein metabolism in addition to its antidiabetic effect.

1. Introduction

Diabetes mellitus is regarded as a syndrome, a collection of disorders that have hyperglycemia as the hallmark. Noninsulin dependent diabetes mellitus is now epidemic in many countries undergoing modernization and industrialization. Diabetes is becoming the third killer of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality [1]. Diabetes is widely recognized as one of the leading causes of death in the world [2]. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Diabetes is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced, thus results in decreased glucose transport into muscle and fat cells, and increased hepatic glucose output. These defects contribute to hyperglycemia, resulting in the impairment of the metabolism of glucose, lipids, proteins and glycoproteins [3]. In the diabetic state, glucose is used by the insulin independent pathways, leading to the synthesis of oligosaccharide moieties of glycoprotein; hexose, hexosamine, fucose, and sialic acid have an important role in protein stability, function, and turnover [4].

Glycoproteins are carbohydrate-linked protein macromolecules found in the cell surface, which is the principle component of animal cell. The oligosaccharide moieties of glycoproteins, hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover [5] membrane transport, cell differentiation and recognition, secretion and absorption of macromolecules and the adhesion of macromolecules to the cell surface [6]. Glycoproteins play a major role in the pathogenesis of diabetes mellitus due to impaired metabolism [7]. Insulin deficiency and high levels of plasma glucose in diabetic condition may result in an increased synthesis of glycoproteins [8]. The level of glycoproteins has been associated with severity and duration of diabetes. At the cell surface or inside the cells, fucose and sialic acid form specific structures, called glycanic chains covalently linked to lipids or proteins. An increase in the biosynthesis and or decrease in the metabolism of glycoproteins could be related to deposition of these materials in the basal membrane of pancreatic cells.

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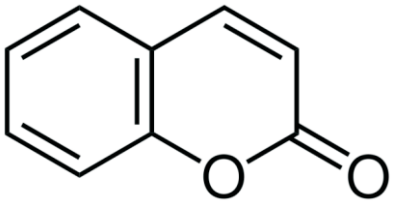
Coumarin (1, 2-benzopyrone) (Figure. 1), naturally occurring phenolic substance composed of fused benzene and α-pyrone rings [9]. It occurs in various plants including tonka beans, sweet clover, fruits (eg. bilberry, cloudberry) green tea, and chicory [10]. The biological and chemical effects of coumarin are antibacterial, antiviral, vasodialatory, antimutagenic, antioxidant [11] and anticancer activity [12]. Coumarin has also been reported to reduce the blood glucose levels [13]. In our previous study, we have demonstrated the efficacy of coumarin on hepatic key enzymes of glucose metabolism in chemical induced type 2 diabetic rats [14].

Figure 1.



Structure of coumarin

To our knowledge, no other biochemical investigation has been carried out on the effect of coumarin in plasma and tissue glycoproteins of experimental diabetic rats. In this view, the present investigation was carried out to study the effect of coumarin on plasma and tissue glycoproteins in rats with streptozotocin and nicotinamide induced diabetes.

2. Materials and Methods

2.1. Animals

Male albino Wistar strain rats (weighing 200-220 g b.w.) were procured from the Central Animal House, Rajah Muthiah Medical College (RMMC), Annamalai University. They were acclimatized to animal house conditions, and fed with standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water ad libitum. The rats 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 the study approved by the ethical committee (Vide.No: 565, 2008), Annamalai University.

2.2. Drug and chemicals

All the chemicals used in this experiment were obtained from Sigma Chemical Company (St Louis, MO, USA), Hi Media (Mumbai, India), and SD-Fine Chemicals (Mumbai, India). All chemicals used were of analytical grade.

2.3. Experimental induction of type 2 diabetes in rats

Non-insulin dependent diabetes mellitus was induced [15] in overnight fasted rats by a single intraperitonial injection of 45 mg/kg body weight STZ, 15 min after the intraperitonial administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (0.1M, pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with plasma glucose concentration more than 250 mg/dl were used for the study.

2.4. Experimental procedure

In the experiment, a total of 24 rats (12 diabetic surviving rats and 12 normal rats) were used. The rats were divided into six in each group. Coumarin was dissolved in vehicle solution (corn oil) and administered orally using an intragastric tube for a period of 45 days.

Group 1: Normal control rats (vehicle treated)
Group 2: Normal rats + coumarin (100 mg/kg b.w)
Group 3: Diabetic rats
Group 4: Diabetic rats + coumarin (100 mg/kg b.w)

At the end of the experimental period, the rats were deprived of food overnight and sacrificed by decapitation. Blood sample was collected in a tube containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma glycoproteins. Liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

2.5.Biochemical assays

2.5.1.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.

2.5.2.Determination of glycoproteins

Plasma and tissue hexose and hexosamine were estimated by the method of Dubois and Gilles [16] and Elson and Morgan [17] with slight modifications by Niebes [18], respectively. Sialic acid and fucose were estimated by the method of Warren [19] and Dische and Shettle [20], respectively.

2.6.Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT) using a statistically software package (SPSS for Windows, V.13.0, Chicago, USA). Results were presented as mean ± S.D. $p<0.05$ were considered as statistically significant [21].

3. Results

In our previous study [14] we have reported that coumarin at 100 mg/kg body weight showed better effect than 25 and 50 mg/kg body weight, therefore the 100 mg/kg body weight was used in this study.

3.1. Effect of coumarin on plasma and tissue glycoproteins

Figure 2 shows the changes in the levels of plasma glycoproteins of control and diabetic rats. Significantly higher levels of glycoproteins were observed in the plasma of the diabetic rats when compared with normal control rats. Treatment with coumarin to diabetic rats resulted in a significant reduction of glycoproteins in the plasma when compared with diabetic control rats.

The levels of liver and kidney glycoprotein of control and experimental rats were shown in Figures 3-6. The levels of

glycoproteins, hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased in diabetic rats. Oral administration of coumarin significantly reversed these changes in the liver and kidney of diabetic rats.

Figure 2.

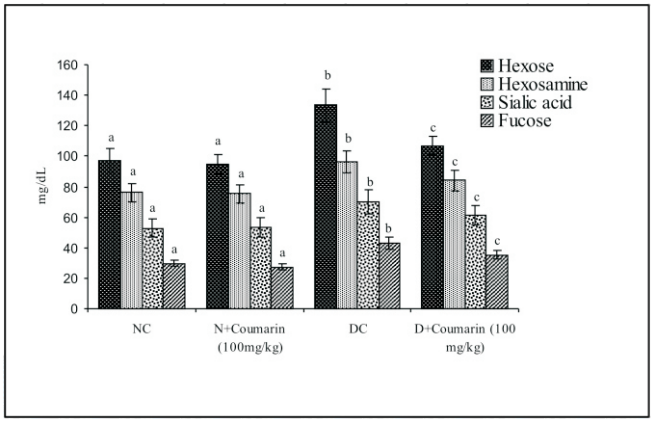


Figure 2. Changes in the levels of plasma glycoproteins in control and experimental rats. NC-normal control, N-normal, DC-diabetic control, D-diabetic; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).

Figure 3.

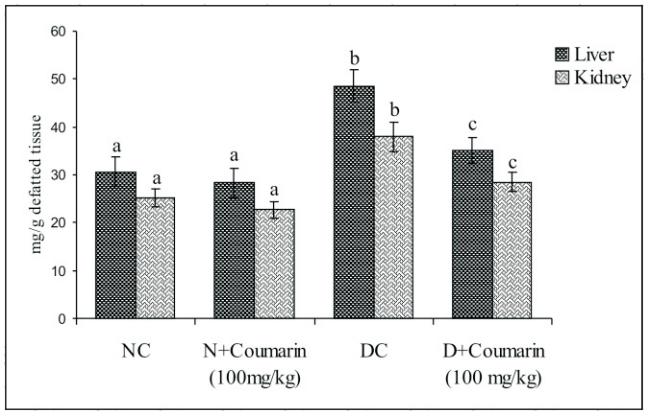


Figure 3. Changes in the levels of tissue hexose in control and experimental rats. NC-normal control, N-normal, DC-diabetic control, D-diabetic; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).

Figure 4.

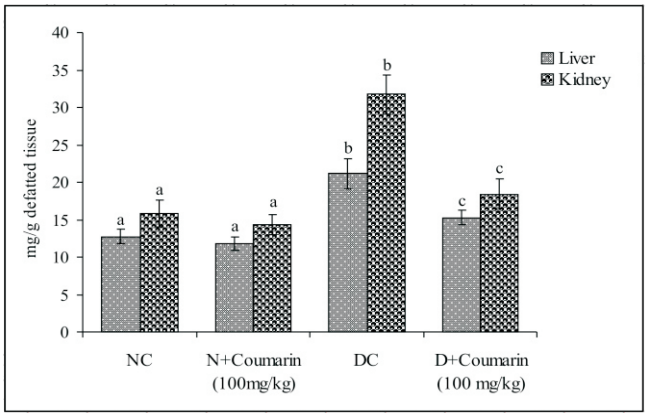


Figure 4. Changes in the levels of tissue hexosamine in control and experimental rats. NC-normal control, N-normal, DC-diabetic control, D-diabetic; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).

Figure 5.

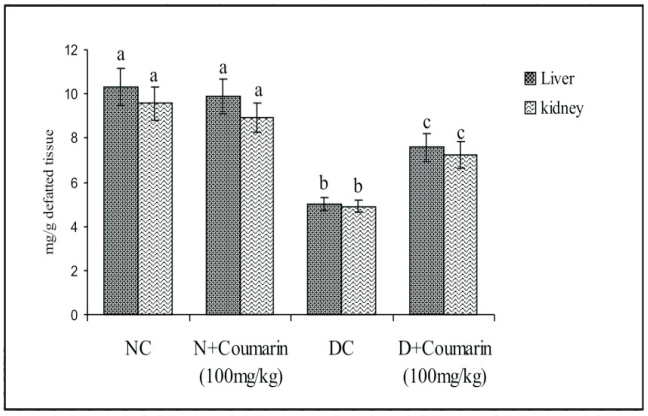


Figure 5. Changes in the levels of tissue sialic acid in control and experimental rats. NC-normal control, N-normal, DC-diabetic control, D-diabetic; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).

Figure 6.

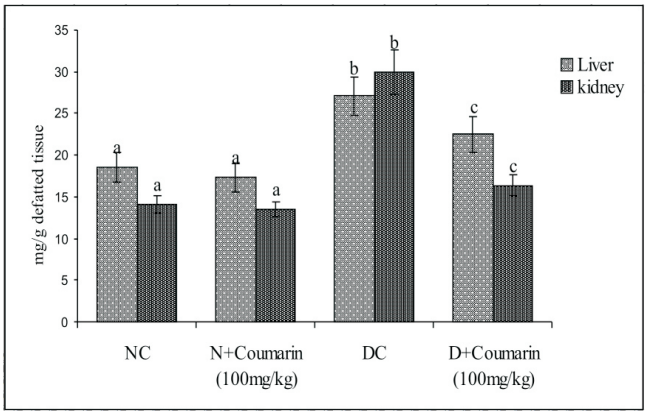


Figure 6. Changes in the levels of tissue fucose in control and experimental rats. NC-normal control, N-normal, DC-diabetic control, D-diabetic; Values are given as mean ±S.D. for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).

4. Discussion

Diabetes mellitus is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemy and abnormalities in carbohydrate, protein and fat metabolism [22]. Masiello et al [15] described a new experimental diabetic model in adult rats by administering STZ and partially protected it with a suitable dose of nicotinamide. Streptozotocin causes diabetes rapid depletion of β-cells and brings about reduction in insulin by release. Insulin has been shown to increase the incorporation of glucose in the rat submaxillary gland [23]. The requirement of insulin for the biosynthesis of the carbohydrate moiety of mucoproteins from glucose is thus evident. In diabetes, synthesis of glycoproteins was decreased because of reduced incorporation of glucose caused by

insulin deficiency. Several studies have emphasized the multiplicity of disturbances affecting the metabolism of carbohydrates, proteins and lipids in diabetes [24, 25]. Carbohydrates seem to play a central role in the development of chronic diabetic complications. Glycation is a non-enzymatic modification of macromolecules induced by the hyperglycemic state during diabetes mellitus.

In this study, we have observed altered levels of hexose, hexosamine, fucose and sialic acid in plasma and tissues of STZ-NA-induced diabetic rats. Glycation is a nonenzymatic reaction of glucose and the saccharide derivatives with proteins, nucleotides and lipids [26]. In hyperglycemia, the reactions occur between reducing sugars and amino groups of proteins to yield a Schiff's-base intermediate. These schiff's base intermediate undergoes rearrangement to form a relatively stable Amadori product. The Amadori product further undergoes a series of reactions through dicarbonyl intermediates to form AGE (advanced glycation end-products). Glycation occurs inside and outside the cells. Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. These effects are countered by protein degradation and renewal.

In extracellular matrix the glycation produces changes in macromolecular structure affecting matrix-matrix and matrix cell interactions associated with decreased elasticity and increased fluid filtration across the arterial wall and endothelial cell adhesion [27]. When the concentration of AGEs increased above a critical level, cell surface AGE receptors become activated.

Abnormalities in the metabolism of glycoproteins are observed in both naturally occurring and experimental diabetes [28]. The increases in plasma glycoprotein components have been reported to be associated with the severity and duration of diabetes. Decreased incorporation of the carbohydrate structure and composition to these in circulation. The vascular complications that involve complex of protein-carbohydrate molecules could contribute to an increase in plasma glycoproteins.

Fucose is member of a group of essential sugars that the body requires for functioning of cell to cell communication and its metabolism appear to be altered in various disease conditions such as diabetes mellitus [29]. Due to increased glycosylation in the diabetic state the fucose levels could be increased. The serum proteins haptoglobin, α-1 acid glycoprotein and α1-antitrypsin are synthesized in liver, the metabolism and synthesis of these proteins may be altered in diabetes leading to changes in serum in the hyperglycemia state accelerates the synthesis of basement membrane components i.e., glycoproteins [30]. The utilization of glucose was depressed by insulin dependent pathways, thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins [31]. Experiments conducted in our laboratory showed elevated levels of fucose in diabetic animals [32]. Our results suggest that the increased fucosylated proteins in diabetic rats could be due to increase in the synthesis and/or decrease in degradation of these proteins.

Sialic acid is a terminal component of the non-reducing end of the carbohydrate chains of glycoproteins and glycolipids, which are essential constituents of many hormones and enzymes present in serum and tissues. Sialic acid is an important constituent for the characteristic changes of transformed cells; the liver is the major site involved in the synthesis of sialic acid and other glycoproteins [8]. The synthesized glycoproteins are made to circulate in blood. There is a pronounced increase in serum rather than in other organs. 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 [33].

The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin independent pathways for the use of glucose 6-phosphate. But the deficiency of insulin during diabetes produces derangement of glycoprotein metabolism, resulting in the thickening of the basal membrane of pancreatic beta cells. In hyperglycemic state, the excess availability of glucose accelerates the synthesis of glucose basement membrane components i.e., glycoproteins [31]. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with glycation. In this context, previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein levels [34]. Administration of coumarin to diabetic rats resulted in a significant reversal of all these changes to near normal.

5. Conclusion

In conclusion, oral administration of coumarin exhibits a protective effect on the carbohydrate moieties of glycoproteins in STZ-nicotinamide induced diabetic rats.

6. References

[1] Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol. 2004; 92: 1-21.

[2] Devendra O, Liu E, Eisenbarth GS. Type 1 diabetes: recent developments. Br Med J. 2004; 328:750-754.

[3] Dhawan D, Bandhu HK, Singh B, Singh A, Nagapal JP. Effect of D-400 (a herbal formulation) on the regulation of glucose metabolism in diabetic rats. Indian J Pharmacol. 1996; 28: 224-226.

[4] Begum N, Moses GPS, Shanmugasundaram KR. Protein bound polysaccharides in human and experimental diabetes. Arogya J Health Sci. 1978; 41:40-144.

[5] Wiese TJ, Dunlap JA, Yorek MA. Effect of L fucose and L-glucose concentration on L fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and a L-fucosidase activity in liver of diabetic rats. Biochim Biophys Acta. 1997; 1: 61-72.

[6] Mittal N, Karur J, Mahmood A. Changes in tubular membrane glycosylation in diabetic, insulin and thyroxin treated rat kidneys. Indian J Exp Biol. 1996; 34: 782-785.

[7] Knecht KT, Bradford BU, Mason RP, Thurman RG. In vivo formation of a free radicals metabolite of ethanol. Mol Pharmacol. 1990; 38: 26-30.

[8] Patti ME, Virkamaki A, Landaker EJ, Kahan CR, ki-Jarvinen H. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early post receptor insulin signaling events in skeletal muscle. Diabetes. 1999; 48: 1562-1571.

[9] Keating GJ, O'kennedy R. In the chemistry and occurrence of coumairns; R O'Kennedy, RD, Thornes (eds). John wiley and Sons, West Sussex, England, 1997, pp 23-64

[10] Egan DA, Kennedy R, Moran E, Thornes RD. The pharmacology, metabolism, analysis and applications of coumarin and coumarin related compounds. Drug Metab Rev. 1990; 22:503-529.

[11] Hoult JR, Paya M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol. 1996; 27: 713-722.

[12] Dexeus FH, Logothetis CJ, Sella A, Fitz K, Amato R, Reuben JM, Dozier N. Phase II study of coumarin and cimitidine in patients with metastatic renal cell carcinoma. J Clin Oncol. 1990; 8: 325-329.

[13] Marles RJ, Farnsworth N. Antidiabetic plants and their active constituents: an update. J Bot Med. 1996; 3: 85-135.

[14] Pari L, Rajarajeswari N. Efficacy of coumarin on hepatic key enzymes of glucose metabolism in chemical induced type 2 diabetic rats. J Chemico-Biol Inter 2009; 181:292-296.

[15] Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998; 47:224-229.

[16] Dubois M, Gilles KA. Methods in Enzymology. Academic Press, New York, 1956, pp 83

17] Elson LA, Morgan WTJ. A colorimetric method for the determination of glucosamine and chondrosamine. Biochem J. 1933; 27:1824-1828.

[18] Niebes P. Determination of enzymes and degradation products of glycosaminoglycans metabolism in the serum of healthy and varicose subjects. Clin Chim Acta. 1972; 42: 399-408.

[19] Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem. 1959; 234:1971-1975.

[20] Dische Z, Shettles LB. A specific color reaction of methyl pentoses and a spectrophotometric micro methed for their determination. J Biol Chem. 1948; 175: 596-603.

[21] Duncan BD. Multiple range test for correlated and heteroscedastic means. Biometrics. 1957; 13:359-364.

[22] Kamtchouing P, Kahpui SM, Djomeni Dzeufiet PD, T'edong L, Asongalem EA, and Dimoa T. Anti-diabetic activity of methnol/methylene chloride stem bark extracts of Terminalia superba and Canarium Schweinfurthii on streptozotocin- induced diabetic rats. J Ethnopharmacol. 2006; 104: 306-309.

[23] Sharma C, Dalferes FR, Radhakrishnamoorthy B, de-Paolo CJ, Berenson GS. Hepatic glycoprotein synthesis in streptozotocin diabetic rats. Biochem Int. 1987; 15-19.

[24] Vlassara H. Recent progress in advanced glycation end products and diabetic complications. Diabetes. 1997; 46: S19-S25.

[25] Bollineni JS, Alluru I, Reddi AS. Heparan sulfate proteoglycan synthesis and its expression are decreased in the retina of diabetic rats. Curr Eye Res. 1997; 16: 127-130.

[26] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414: 813-820.

[27] Thornalley PJ. Glycation and/or polyol pathway inducing complications. Encyclopedia of Endocrine Dis. 2004; 2: 257-278.

[28] Latha M, Pari L. Effect of an aqueous extract of Scoparia dulcis on plasma and tissue glycoproteins in streptozotocin induced diabetic rats. Pharmazie. 2005; 60: 151-154.

[29] Mondoa EI, Kitei M. The new healing science of glyconutrients. In: Sugars that Heal, Ballantine Publishing, New York, 2001

[30] Radhakrishnamoorthy B, Berenson GS. Structure of glycopeptides from a glycoprotein from bovin aorta, J Biol Chem. 1973; 248: 2000-2010.

[31] Spiro RG, Spiro MJ. Effect diabetes on the biosynthesis of the renal glomerular basement membrane. Studies on the glycosyl transferase. Diabetes. 1971; 20: 641-648.

[32] Pari L, Murugan P. Changes in Glycoprotein Components in Streptozotocin - Nicotinamide Induced Type 2 Diabetes: Influence of Tetrahydrocurcumin from Curcuma longa. Plant Food Hum Nutr. 2007; 62: 25-29.

[33] Schiller S, Dorfman A. The metabolism of mucopolysaccharides in animals. The influence of insulin. J Biol Chem. 1957; 227:625-632.

[34] Pari L, Karthikesan K. Protective role of tetrahydrocurcumin and chlorogenic acid on glycoprotein changes in streptozotocin-nicotinamide-induced diabetic rats. J Pharm Sci & Res. 2009; 4: 173-180.