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Effect of a Herbal Drug, Cogent db on Plasma and Tissue Glycoproteins in Alloxan-Induced Diabetic Rats

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Abstract: Cogent db, a poly herbal drug, was investigated for its beneficial effect in diabetic rats on derangement in glycoprotein components. Diabetes was induced in male albino Wistar rats by a single intraperitoneal injection of alloxan (150 mg kg^{-1}) and the animals were divided into 5 groups as follows: Group 1: Normal untreated rats, Group 2: Normal rats treated with Cogent db (450 mg kg^{-1}), Group 3: Diabetic control rats, Group 4: Diabetic rats treated with Cogent db (450 mg kg^{-1}) and Group 5: Diabetic rats treated with glibenclamide (600 µg kg^{-1}). The effect of Cogent db on blood glucose, plasma insulin, urine sugar, plasma and tissue glycoproteins studied was in comparison to glibenclamide, a standard reference drug. The levels of blood glucose, urine sugar, 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, fucose in the liver and kidney of diabetic rats. Oral administration of Cogent db to diabetic rats for 45 days significantly decreased levels of blood glucose, urine sugar and plasma glycoproteins. On the other hand, the levels of plasma insulin and tissue sialic acid were increased while the levels of tissue hexose, hexosamine and fucose were near normal. Cogent db was more effective than glibenclamide in restoring the values of these parameters. It is likely that the changes in glycoprotein metabolism induced by hyperglycaemia will have biological and possibly pathological importance in the development of diabetic complications. The present investigation indicates that Cogent db treatment possesses a significant beneficial effect on glycoproteins in addition to its antidiabetic action.

Key words: Glycoproteins, alloxan-induced diabetes, cogent db, herbal drug, antidiabetic effect, plasma insulin

INTRODUCTION

Diabetes is a complex multisystemic disorder characterized by a relative or absolute insufficiency of insulin secretion or concomitant resistance of the metabolic actions of insulin on target tissues. The condition affects the metabolism of carbohydrate, lipid, protein and glycoprotein leading to structural changes in a range of cells and subsequently leading to long-term complications of diabetes (Garau *et al.*, 2003; Wild *et al.*, 2004). Hyperglycaemia, the primary clinical manifestation in diabetes, is associated with the development of certain complications of diabetes. Brief episodes of hyperglycaemia cause tissue damage by mechanisms involving repeated acute changes in cellular metabolism. However, exposure to high glucose also causes cumulative changes in long-lived macromolecules (Brownlee, 2001; Rolo and Palmeira, 2006).

Glycoproteins are carbohydrate linked protein macromolecules found in the cell surface, which is the principal component of animal cells. It is well documented that the oligosaccharide moieties of

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Table 1: Composition and concentration of Cogent db tablet

Botanical name	Family	Weight (mg)
Azardirachta indica	Meliaceae	360
Phyllanthus emblica	Euphorbiaceae	85
Treminalia bellerica	Combretaceae	85
Treminalia chebula	Combretaceae	85
Tribulus terestris	Zygophyllaceae	120
Trigonella foenum graecum	Apiaceae	15
Curcuma longa	Zingiberaceae	95
Syzygium cumini	Myrtaceae	240
Rotula aquatica	Lythiraceae	120

glycoproteins: Hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover (Wiese *et al.*, 1997; Wu, 2003). The knowledge concerning the involvement of glycoproteins in diabetic complications led many authors to closely observe the glycoprotein changes in diabetic patients. Rahman *et al.* (1990) found a significant rise in serum mucoprotein, hexosamine, sialic acid and fucose in diabetic patients and observed that with increasing severity of diabetes, there was a parallel rise in glycoprotein. Analysis of human diabetic basement membrane showed both an absolute increase in glycoprotein and a different composition, compared with normal human basement membrane. Spiro (1963) has suggested that in diabetes there may be defects in glycolysis resulting in the shunting of glucose from insulin-dependent pathways to the biosynthesis of glycoprotein. Shaw *et al.* (1968) explored this concept by measuring the concentration in serum of fucose, a sugar nucleotide concerned with the incorporation of carbohydrates into glycoproteins. In diabetic state, glucose is utilized by insulin independent pathways leading to the synthesis of glycoproteins and even mild deficiency of insulin influences thickening of basement membrane (Konukoglu *et al.*, 1999).

Recently, search for appropriate antihyperglycaemic agents has been focused on plants used in traditional medicine because of leads provided by natural products that may be better treatment than currently used drugs (Rates, 2001; Mazunder *et al.*, 2005; Singab *et al.*, 2005). In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual. Their cumulative effect increases the efficacy of the drug in curing the diseases (Palani *et al.*, 1999). Cogent db is a poly herbal antidiabetic drug comprising of nine various plant ingredients whose composition and concentrations are given in Table 1. Cogent db is extensively used for the treatment of diabetes mellitus. Previous studies from this laboratory proved the antidiabetic and antihyperlipidaemic effects of Cogent db in diabetic rats (Pari and Saravanan, 2002; Saravanan *et al.*, 2002; Saravanan and Pari, 2003). However, no other biochemical investigations had been carried out on the effect of Cogent db in diabetic rats on glycoproteins status, so the present investigation was carried out to study the effect of Cogent db on plasma and tissue glycoproteins in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Test Drug and Chemicals

Cogent db, the herbal drug was a gift from Cybele Herbal Laboratories, Kerala, India. Alloxan monohydrate was purchased from BDH chemicals Ltd., Poole, England. Enzyme Linked Immunosorbant Assay (ELISA) kit for insulin assay was purchased from Boehringer Mannheim, Germany. All other biochemicals used in this experiment were purchased.

Drug Administration

Cogent db tablets were suspended in distilled water and administered orally through intragastric tube at a dose of 450 mg kg^{-1} body weight.

Experimental Animals

Adult male albino rats of Wistar strain weighing approximately 180-200 g were obtained from Central Animal House, Department of Experimental Medicine, Faculty of Medicine, Rajah Muthiah Medical College, Annamalai University. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of $22\pm2°C$ and humidity of 45-64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. All animal experiments were approved by the Ethical Committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, India. The rats received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institutes of Health.

Experimental Induction of Diabetes in Rats

Rats were injected intraperitoneally with freshly prepared solution of alloxan monohydrate in normal saline at a dose of 150 mg kg^{-1} body weight (Katsumata *et al.*, 1999). Because alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (5-10 mL) orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia (Gupta *et al.*, 1984). After 2 weeks, rats with moderate diabetes that exhibited glycosuria and hyperglycaemia (i.e., blood glucose concentration 200-300 mg dL^{-1}) were taken for the experiment.

Experimental Design

In the experiment, a total of 30 rats were used. The rats were divided into 5 groups of 6 rats each as follows:

Group 1: Normal untreated rats.

Group 2: Normal rats received Cogent db (450 mg kg^{-1}) in aqueous solution orally for 45 days.

Group 3: Diabetic control rats

Group 4: Diabetic rats received Cogent db (450 mg kg^{-1}) in aqueous solution orally for 45 days (Pari and Saravanan, 2002).

Group 5: Diabetic rats received glibenclamide (600 µg kg^{-1}) in aqueous solution orally for 45 days (Pari and Saravanan, 2002).

Since diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycaemic/antihyperglycaemic agents for a longer duration. In addition, this application would be beneficial to reveal the late onset activity profile of the agent (Sezik *et al.*, 2005). Therefore an experiment was planned to assess the effect of Cogent db for a period of 45 days in alloxan-induced diabetic rats. Cogent db and glibenclamide treatments were started after 15 days of alloxan injection. No detectable irritation or restlessness was observed after each drug or vehicle administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animals after the drug administration. Throughout the experimental period, the body weight, food and fluid intake were monitored. At the end of 45 days, all the rats were killed by decapitation (Pentobarbitone sodium) anesthesia (60 mg kg^{-1}). Blood was collected in heparin-coated tubes and centrifuged at 1,000 g for 15 min at 4°C. Liver and kidney were dissected out, washed in ice cold saline, patted dry and weighed.

Measurement of Blood Glucose and Urine Sugar

Blood glucose was determined by the O-toluidine method (Sasaki *et al.*, 1972). Urine glucose was assessed in fresh urine using glucose indicator sticks (Boerhinger Mannheim, Germany).

Quantitative Determination of Plasma Insulin

The plasma insulin was assayed by ELISA method (Awareness Technologies, USA) using Boehringer Mannheim kit.

Estimation of Glycoproteins

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, $0.1N$ NaOH was added. The aliquots were used for fucose, hexose and hexosamine estimation. Hexose was estimated by the method of Niebes (1972). Hexosamine was estimated by the method of Elson and Morgan (1933) with slight modifications of Niebes (1972). Sialic acid and fucose were determined by the method of Warren (1959) and Dische and Shettle (1948).

Statistical Analysis

All data were expressed as mean±SD of number of experiments. The statistical significance was evaluated by one-way Analysis of Variance (ANOVA), using SPSS version 9.5 (SPSS, Cary, NC, USA). Individual comparisons were obtained by Duncan's Multiple Range Test (DMRT) (Duncan, 1957). A p-value of <0.05 was considered a significant difference between groups.

RESULTS

A significant increase in the levels of blood glucose, urine sugar and decrease in the level of plasma insulin was observed in alloxan-induced diabetic rats when compared with corresponding control rats. Administration of Cogent db and glibenclamide to diabetic rats tends to bring these changes to near normalcy (Table 2). The effect of Cogent db (450 mg kg^{-1}) was found to be greater than that of glibenclamide (600 µg kg^{-1}).

The diabetic rats showed a significant increase in the level of food and fluid intake when compared with control rats. Administration of Cogent db and glibenclamide to diabetic rats significantly reversed these changes in the level of food and fluid consumption (Table 3).

A marked elevation in the levels of hexose and hexosamine and fucose was observed in plasma, liver and kidney of diabetic rats when compared to those of control rats. A significant rise in the level

Table 2: Effect of Cogent db on changes in the levels of blood glucose, plasma insulin, urine sugar and body weight in normal and experimental rats

| Groups | Changes in body weight (g) | | Blood glucose (mg dL^{-1}) | Plasma insulin (µU mL^{-1}) | Urine sugar |
	Initial	Final			
1	198.5±9.20	210.2±10.3^a	74.45±5.31^a	25.30±2.52^a	Nil
2	195.4±10.0	208.6±11.2^a	63.26±4.30^b	25.55±2.40^a	Nil
3	191.3±10.3	150.5±10.0^b	230.50±13.6^c	11.10±1.01^b	+++
4	188.1±9.50	200.3±8.70^a	83.40±6.70^d	22.61±2.10^c	Nil
5	184.2±8.80	192.6±9.40^c	91.20±6.93^e	20.83±1.90^d	Trace

Values are given as mean±SD from six rats in each group; Values not sharing a common superscript letter(s) differ significantly at p<0.05 (DMRT); +++: Indicates more than 2% sugar

Table 3: Effect of Cogent db on changes in the levels of food intake and fluid intake in normal and experimental rats

| Groups | Food intake (g rat^{-1} day^{-1}) | | Fluid intake (mL rat^{-1} day^{-1}) | |
	Before treatment	After treatment	Before treatment	After treatment
1	16.3±0.9	15.6±1.0^a	72.5±8.50	70.0±9.2^a
2	18.7±1.0	16.5±0.8^a	75.2±8.00	73.5±7.8^a
3	41.2±3.3	58.9±6.8^b	153.4±16.2	168.0±28.3^b
4	25.6±1.8	30.0±2.3^c	123.1±9.50	80.5±7.6^c
5	26.4±2.0	33.3±1.9^c	129.3±9.00	93.0±8.9^c

Values are given as mean±SD from six rats in each group; Values not sharing a common superscript letter(s) differ significantly at p<0.05 (DMRT)

Table 4: Effect of Cogent db on changes in the levels of plasma and tissue glycoproteins in normal and experimental rats

Glycoproteins components	Normal	Normal + cogent db (450 mg kg⁻¹)	Diabetic control	Diabetic + cogent db (450 mg kg⁻¹)	Diabetic + cogent db (600 µg kg⁻¹)
Hexose					
Plasma (mg dL⁻¹)	95.20±4.03[a]	93.80±3.88[a]	136.90±6.95[b]	103.50±4.80[c]	110.40±4.93[d]
Liver (mg g⁻¹ tissue)	30.43±2.46[a]	28.56±2.12[a]	49.89±3.15[b]	33.47±2.19[c]	37.90±2.74[d]
Kidney (mg g⁻¹ tissue)	25.30±1.41[a]	23.60±1.24[a]	41.60±2.50[b]	29.00±1.82[c]	32.80±2.00[d]
Hexosamine					
Plasma (mg dL⁻¹)	74.20±3.95[a]	72.31±3.52[a]	99.10±6.10[b]	83.14±3.62[c]	89.33±3.75[d]
Liver (mg g⁻¹ tissue)	9.06±0.54[a]	8.80±0.38[a]	15.25±0.92[b]	10.67±0.61[c]	12.16±0.72[d]
Kidney (mg g⁻¹ tissue)	14.01±0.96[a]	13.32±0.87[a]	27.65±1.94[b]	17.74±1.34[c]	20.30±1.45[d]
Sialic acid					
Plasma (mg dL⁻¹)	54.03±2.65[a]	52.61±2.50[a]	73.02±4.10[b]	58.94±2.49[c]	62.08±3.28[d]
Liver (mg g⁻¹ tissue)	8.25±0.43[a]	8.32±0.40[a]	4.73±0.25[b]	7.50±0.31[c]	6.88±0.23[d]
Kidney (mg g⁻¹ tissue)	7.88±0.44[a]	8.02±0.45[a]	4.00±0.20[b]	7.10±0.35[c]	6.45±0.29[d]
Fucose					
Plasma (mg dL⁻¹)	29.36±1.73[a]	27.24±1.60[a]	42.10±2.30[b]	32.94±1.70[c]	36.98±1.96[d]
Liver (mg g⁻¹ tissue)	15.26±1.19[a]	13.80±1.24[a]	26.53±2.16[b]	17.35±1.25[c]	20.20±1.54[d]
Kidney (mg g⁻¹ tissue)	13.30±1.00[a]	11.90±0.82[a]	25.24±1.72[b]	15.65±1.17[c]	18.35±1.42[d]

Values are given as mean±SD from six rats in each group; Values not sharing a common superscript letter(s) differ significantly at p<0.05 (DMRT)

of sialic acid was seen in plasma and liver, whereas a significant decrease was seen in kidney of diabetic rats in comparison with control rats (Table 4). Oral administration of Cogent db and glibenclamide significantly restored the altered glycoprotein components of diabetic rats.

DISCUSSION

Alloxan has been widely used to induce diabetes mellitus in experimental animal models allowing investigation of hypoglycaemic agents in the treatment of diabetes (Reyes *et al.*, 2006). It causes diabetes through its ability to destroy the insulin-producing β-cells of the pancreas. *In vitro* studies have shown that alloxan is selectively toxic to pancreatic β-cells, leading to the induction of cell necrosis. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of β-cells (Jorns *et al.*, 1997; Szkudelski, 2001). Alloxan treatment consistently produced symptoms characteristic of diabetes including hyperglycaemia, decreased insulin levels, polyuria and weight loss. Oral administration of Cogent db to diabetic rats for 45 days caused a significant antihyperglycaemic effect. The capacity of Cogent db to significantly decrease the elevated blood glucose close to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. Moreover, it indirectly indicates that the antidiabetic effect of Cogent db is partly due to a release of insulin from the existing cells of the pancreas (Pari and Saravanan, 2002; Saravanan *et al.*, 2002; Saravanan and Pari, 2003). The possible mechanism of action of Cogent db could be correlated with the reminiscent effect of the hypoglycaemic sulphonylureas that promote insulin secretion by closure of K⁺-ATP channels, membrane depolarization and stimulation of Ca²⁺ influx, an initial key step in insulin secretion.

The diabetic rats showed a significant decrease in body weight although they consumed a larger quantity of food than did the non-diabetic animals. Rasch (1980) reported that rise in body weight was far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. The mobilization of protein and fat stores may be responsible for the body weight loss noted in diabetic rats, since food conversion efficiency of diabetic animals was lower than that of the non-diabetic animals. Administration of Cogent db significantly improved the body weight in diabetic rats, which may be due to its protective effect in controlling muscle wasting (i.e., gluconeogenesis) and may also be due to the improvement in insulin secretion and glycaemic control.

Generalized abnormalities in glycoproteins metabolism is observed in both naturally occurring and experimental diabetes. Changes in glycoprotein concentration and its composition of various proteins in diabetic patients have been correlated with hyperglycaemia which in turn causes early functional alterations in peripheral nerves, kidney and retina that antedate the development of characteristic diabetic pathology in these susceptible sites (Rahman *et al.*, 1990). Berenson *et al.* (1972) reported that streptozotocin diabetic rats exhibited a significant modification in the connective tissue macromolecules. 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.

Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in plasma (Radhakrishnamurthy and Berenson, 1973). Previous reports suggest that plasma concentrations of glycoproteins are significantly increased in diabetic animals (Johnson and Wales, 1976; McMillan, 1989). The elevated levels of plasma glycoprotein components in the present study may be due to secretion or shedding from cell membrane glycoconjugates into the circulation.

The liver is primarily responsible for producing a large amount of glycoproteins present in blood. The biosynthesis of the carbohydrate moieties of glycoprotein forms the insulin independent pathways for the utilization of glucose-6-phosphate. But the deficiency of insulin during diabetes produces derangement of glycoprotein metabolism, resulting in the thickening of basal membrane. The increased availability of glucose in the diabetic state accelerates the synthesis of basement components (i.e., glycoproteins) and thereby enhancing the formation of hexose, hexosamine and fucose for the accumulation of glycoproteins (Spiro and Spiro, 1971; Patti *et al.*, 1999).

Alterations of enzymatic glycosylation processes detected as changes in sialic acid and fucose tissue contents have been previously reported in diabetic patients and animals (Rellier *et al.*, 1999). The elevation of serum glycoprotein levels with a disproportionate increase in the level of serum protein-bound fucose has been reported. Studies have also indicated that serum and hepatic fucosyltransferase and fucosidase activities are increased in streptozotocin-induced diabetic rats (Wiese *et al.*, 1997). In diabetes, three serum proteins (haptoglobin, α1-acid glycoprotein and α1-antitrypsin) synthesized in the liver are mainly responsible for the increase in bound fucose levels (Mobley *et al.*, 1972; McMillan, 1972). Present findings also suggest that the increased fucosylated proteins in diabetic rats could be due to an increase in the synthesis and/or decrease in degradation of these proteins.

In this study, the serum sialic acid level was found to be significantly increased but tissue sialic acid was decreased in diabetic rats. The cleavage of sialic acid residue from circulatory and membrane glycoproteins might be the cause of high serum levels. Alterations in membrane protein glycosylation may also be important in membrane protein turnover. Since carbohydrate groups such as sialic acid render protection against proteolysis of glycoproteins, a decrease in superficial carbohydrate moieties might increase proteolysis and hence membrane protein degradation (Jacobs, 1981; Mazzanti *et al.*, 1997). Moreover, a decrease in sialic acid will induce a decrease in the negative electrostatic charge of endothelial cells that may participate to the hyperagregability of circulating cells to the vascular endothelium (Rellier *et al.*, 1999).

The possibility of a general decrease in sialylation of proteins in diabetes is further supported by other studies including the decreased activity of hepatic enzymes associated with sialic acid synthesis in diabetic rats (Pickup *et al.*, 1995). The decrease in the content of sialic acid in tissues may also be due to its utilization for the synthesis of fibronectin, which contain sialic acid residues in the core structure. The synthesis of fibronectin was reported to increase significantly in various tissues of diabetic animals and patients (Noh *et al.*, 2002; Chen *et al.*, 2002; Isono *et al.*, 2002). Administration of Cogent db to diabetic rats significantly reversed all these changes to near normal levels.

The antidiabetic action of Cogent db, which is mediated via an enhancement of insulin action, as it is evidenced by the increased level of insulin in diabetic rats treated with Cogent db, may be responsible for the reversal of changes in carbohydrate moieties of glycoprotein. In conclusion, the administration of Cogent db to diabetic rats has a beneficial effect on the carbohydrate moieties of glycoproteins in addition to its antidiabetic effect. Present results revealed the potential therapeutic value of Cogent db as an alternative medicine for the better control, management and prevention of diabetes mellitus progression. Further biochemical and pharmacological investigations are underway to elucidate the precise mechanism of action of this drug.

REFERENCES

Berenson, G.S., B. Radhakrishnamurthy, E.R. Dalferes, H. Ruiz and S.R. Srinivasan, 1972. Connective tissue macromolecular changes in rats with experimentally induced diabetes and hyperinsulinism. Diabetes, 21: 733-743.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813-820.

Chen, H.C., J.Y. Guh, S.J. Shin and Y.H. Lai, 2002. Pravastatin suppress superoxide and fibronectin production of glomerular mesangial cells induced by oxidized-LDL and high glucose. Atherosclerosis, 160: 141-146.

Dische, Z. and L.B. Shettle, 1948. A specific color reaction of methyl pentoses and a spectrophotometric micro method for their determination. J. Biol. Chem., 175: 596-603.

Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 359-364.

Elson, L.A. and W.T.J. Morgan, 1933. A colorimetric method for the determination of glucosamine and chondrosamine. Biochem. J., 27: 1824-1828.

Folch, J., M. Lees and S.G.H. Solane, 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., 26: 497-509.

Garau, C., E. Cummings, D.A. Phoenix and J. Singh, 2003. Beneficial effect and mechanism of action of *Momordica charantia* in the treatment of diabetes mellitus: A mini review. Int. J. Diab. Metab., 11: 46-55.

Gupta, M.P., N.G. Solis, M. Esposito and S. Sanchez, 1984. Hypoglycemic activity of *Neurolaena lobata* (L.). J. Ethnopharmacol., 10: 323-327.

Isono, M., S. Chen, S.W. Hong, M.C. Iglesias-de la Cruz and F.N. Ziyadeh, 2002. Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-beta-induced fibronectin in mesangial cells. Biochem. Biophys. Res. Commun., 296: 1356-1365.

Jacobs, L.R., 1981. Alterations in labeling of cell-surface glycoproteins from normal and diabetic rat intestinal microvillous membranes. Biochim. Biophys. Acta, 649: 155-161.

Johnson, A. and J.K. Wales, 1976. Blood glycoprotein levels in diabetes mellitus. Diabetologia, 12: 245-250.

Jorns, A., R. Munday, M. Tiedge and S. Lenzen, 1997. Comparative toxicity of alloxan, N-alkyl-alloxans and ninhydrin to isolated pancreatic islets *in vitro*. J. Endocrinol., 155: 283-293.

Katsumata, K., Y. Katsumata, T. Ozawa and K. Katsumata, 1999. Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan diabetic rats. Horm. Metab. Res., 25: 125-126.

Konukoglu, D., O. Serin, T. Akcay and H. Hatemi, 1999. Relationship between diabetic angiopathic complications and serum total and lipid associated sialic acid concentrations. Med. Sci. Res., 27: 53-55.

Mazunder, U.K., M. Gupta and Y. Rajeshwar, 2005. Antihyperglycemic effect and antioxidant potential of *Phyllanthus niruri* (Euphorbiaceae) in streptozotocin induced diabetic rats. Eur. Bull. Drug Res., 13: 15-23.

Mazzanti, L., R.A. Rabini and E. Salvolini, 1997. Sialic acid, diabetes and aging: A study on the erythrocyte membrane. Metabolism, 46: 59-61.

Mobley, P.W., R.P. Metzger, J.G. Guilmette and A.N. Wick, 1972. The effect of fasting, alloxan diabetes and streptozotocin diabetes on rat liver D-arabinose (L-fucose) dehydrogenase activity. Biochem. Med., 6: 178-183.

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

Noh, H., H. Ha and M.R. Yu, 2002. High glucose increases inducible NO production in cultured rat mesangial cells. possible role in fibronectin production. Nephron, 90: 78-85.

Palani, V., R.K. Senthilkumaran and S. Govindasamy, 1999. Biochemical evaluation of antitumor effect of Muthu Marunthu (a herbal formulation) on experimental fibrosarcoma in rats. J. Ethnopharmacol., 65: 257-265.

Pari, L. and G. Saravanan, 2002. Antidiabetic effect of Cogent db, a herbal drug in alloxan induced diabetes mellitus. Comp. Biochem. Physiol. Toxicol. Pharmacol., 131: 19-25.

Patti, M.E., A. Virkamai, E.J. Landaker, C.R. Kahn and H. Jarvinen, 1999. Activation of the hexosamine pathway by glucosamine *in vivo* induces insulin resistance of early post receptor insulin signalling events in skeletal muscles. Diabetes, 48: 1562-1571.

Pickup, J.C., C. Day and C.J. Bailey, 1995. Plasma sialic acid in animal models of diabetes mellitus: Evidence for modulation of sialic acid concentrations by insulin deficiency. Life Sci., 57: 1383-1391.

Radhakrishnamurthy, B. and G.S. Berenson, 1973. Structure of glycopeptides from a glycoprotein from *Bovine aorta*. J. Biol. Chem., 248: 2000-2010.

Rahman, M.A., G. Zafar and A.S. Shera, 1990. Changes in glycosylated proteins in long-term complications of diabetes mellitus. Biomed. Pharmacother., 44: 229-234.

Rasch, R., 1980. Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Diabetologia, 18: 413-416.

Rates, S.M., 2001. Plants as source of drugs. Toxicon, 39: 603-613.

Rellier, N., D. Ruggiero-Lopez, M. Lecomte, M. Lagarde and N. Wiernsperger, 1999. *In vitro* and *in vivo* alterations of enzymatic glycosylation in diabetes. Life Sci., 64: 1571-1583.

Reyes, B.A.S., N.D. Bautista and N.C. Tanquilut, 2006. Anti-diabetic potentials of *Momordica charantia* and *Andrographis paniculata* and their effects on estrous cyclicity of alloxan-induced diabetic rats. J. Ethnopharmacol., 105: 196-200.

Rolo, A.P. and C.M. Palmeira, 2006. Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. Toxicol. Applied Pharmacol., 212: 167-178.

Saravanan, G., L. Pari and S. Venkateswaran, 2002. Effect of Cogent db, a herbal drug on plasma insulin and enzymes of glucose metabolism in experimental diabetes. Diabetes Obes. Metab., 4: 394-398.

Saravanan, G. and L. Pari, 2003. Effect of Cogent db, a herbal drug on serum and tissue lipid metabolism in experimental hyperglycaemic rats. Diabetes Obes. Metab., 5: 156-162.

Sasaki, T., S. Matsy and A. Sonae, 1972. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku, 1: 346-353.

Sezik, E., M. Aslan, E. Yesilada and S. Ito, 2005. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. Life Sci., 76: 1223-1238.

Shaw, R.A., L.J. Kryston and L.C. Mills, 1968. Plasma fucose and fucose tolerance in diabetes mellitus. Ann. NY Acad. Sci., 148: 787-795.

Singab, A.N., H.A. El-Beshbishy, M. Yonekawa, T. Nomura and T. Fukai, 2005. Hypoglycemic effect of Egyptian *Morus alba* root bark extract: Effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. J. Ethnopharmacol., 100: 333-338.

Spiro, R.G. and M.J. Spiro, 1971. Effect of diabetes on the biosynthesis of the renal glomerular basement membrane. Studies on the glycosyl transferase. Diabetes, 20: 641-648.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiol. Res., 50: 536-546.

Warren, L., 1959. Thiobarbituric acid assay of sialic acid. J. Biol. Chem., 234: 1971-1975.

Wiese, T.J., J.A. Dunlap and M.A. Yorek, 1997. Effect of L-fucose and D-glucose concentration on L-fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and α-L-fucosidase activity in liver of diabetic rats. Biochim. Biophys. Acta, 1335: 61-72.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.

Wu, A.M., 2003. Carbohydrate structural units in glycoproteins and polysaccharides as important ligands for Gal and GalNAc reactive lectins. J. Biomed. Sci., 10: 676-688.