JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1373–1383

Received May 2, 2006
Accepted June 19, 2006
DOI 10.1211/jpp.58.10.0011
ISSN 0022-3573

Protective effect of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats

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Abstract

The protective role of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins in streptozotocin-induced diabetic rats has been studied. A single intraperitoneal injection of streptozotocin (50 mg kg^{-1}) to rats led to a significant ($P < 0.05$) increase in the levels of lipids (cholesterol, triglycerides, free fatty acids and phospholipids) in plasma and tissues (liver, kidney, heart and brain). The levels of low density and very low density lipoprotein (LDL and VLDL, respectively) cholesterol were increased, whereas the levels of high density lipoprotein (HDL) cholesterol were decreased significantly ($P < 0.05$) in plasma. The activity of 3-hydroxy 3-methylglutaryl coenzyme A (HMG CoA) reductase increased significantly ($P < 0.05$) in liver, kidney and heart, and the activity of lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT) decreased significantly ($P < 0.05$) in the plasma of diabetic rats. Streptozotocin injection also increased the levels of glycoproteins such as hexose, hexosamine, fucose and sialic acid in plasma, liver and kidney. Oral administration of rutin to streptozotocin-induced diabetic rats significantly ($P < 0.05$) decreased the levels of lipids in plasma and tissues. The levels of plasma HDL-cholesterol increased and the levels of LDL- and VLDL-cholesterol decreased significantly ($P < 0.05$). The activity of HMG CoA reductase decreased in the tissues and the activity of plasma LPL and LCAT increased significantly ($P < 0.05$). The levels of glycoproteins were found to be significantly ($P < 0.05$) decreased in plasma, liver and kidney of rutin-treated diabetic rats. Rutin administration to normal rats did not exhibit any significant ($P < 0.05$) changes in any of the parameters studied. In conclusion, the beneficial effect of rutin on lipids, lipoproteins, lipid metabolizing enzymes and glycoproteins could be due to its antioxidant property.

Introduction

Cardiovascular diseases constitute the main causes of morbidity and mortality in diabetic patients. Diabetes mellitus is very often associated with a marked increase in well known parameters of cardiovascular risk including hypertension, hypertriglyceridaemia and low levels of high density lipoprotein (HDL)-cholesterol (Garber 2002). The goals of managing diabetes mellitus are to optimize the control of blood glucose, reduce the effects of oxidative stress and normalize disturbances in lipid metabolism that could predispose patients to cardiovascular complications.

Under diabetic conditions, reactive oxygen species are produced mainly through glycation reactions, which occur in various tissues and may play an important role in the development of diabetic complications (Baynes 1991). Advanced glycation end products (AGEs) modify galactose, fucose and sialic acid contents of specific cellular glycoproteins (Rellier et al 1999).

Flavonoids occur naturally in fruits, vegetables, nuts, seeds, flowers and barks and are an integral part of the human diet (Middleton et al 2000). Flavonoid consumption is significantly inversely related to mortality from coronary artery disease. Regular ingestion of flavonoid-containing foods may protect against death from coronary artery disease in elderly men (Middleton et al 2000). Quercetin is one of the most common native flavonoids occurring mainly in its glycosidic form such as rutin (Havsteen 1983). Quercetin and rutin are the flavonoids most abundantly consumed in foods. In Western diets the richest sources

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of quercetin glycosides are onions (347 mg kg^{-1}), apples (36 mg kg^{-1}), tea (20 mg kg^{-1}) and red wine (11 mg kg^{-1}) (Hertog et al 1993).

Previously, we reported that rutin decreased plasma glucose, glycosylated haemoglobin, plasma thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides, and increased the levels of plasma insulin, C-peptide, total haemoglobin and plasma nonenzymic antioxidants in streptozotocin-induced diabetic rats (Kamalakkannan & Stanely Mainzen Prince 2006a). Administration of rutin to streptozotocin-induced diabetic rats decreased the activity of glucose 6-phosphatase and fructose 1,6-bisphosphatase and increased the activity of hexokinase in the tissues. Rutin treatment lowered the concentration of lipid peroxidative products (TBARS and hydroperoxides) and improved the antioxidant system in the diabetic pancreas (Stanely Mainzen Prince & Kamalakkannan 2006). Rutin decreased the content of hydroxyproline and collagen in the kidney of streptozotocin-induced diabetic rats. The activity of matrix metalloproteinases (MMPs) increased and the levels of tissue inhibitors of metalloproteinases (TIMPs) decreased in the kidney of streptozotocin-induced diabetic rats (Kamalakkannan & Stanely Mainzen Prince 2006b). Rutin also decreased the lipid peroxidative products and improved the antioxidants in streptozotocin-induced diabetic liver, kidney and brain (Kamalakkannan & Stanely Mainzen Prince 2006c). The antioxidant effect of rutin in isoprenaline-induced myocardial infarction in rats was also reported (Karthick & Stanely Mainzen Prince 2006).

A preliminary report by Krishna et al (2005) showed that cyclodextrin complexes of rutin-treated streptozotocin-diabetic rats significantly decreased serum triglycerides and cholesterol. As there are no available reports detailing the role of rutin on lipid metabolism in streptozotocin-induced diabetes, this study has examined the possible effects of rutin on lipids in plasma and tissues, and lipoproteins in plasma and marker enzymes of lipid metabolism. In addition, the effect of rutin on the levels of glycoproteins such as hexose, hexosamine, fucose and sialic acid in plasma, liver and kidney of normal and streptozotocin-induced diabetic rats were studied.

Materials and Methods

Animals

Male albino Wistar rats (150–180 g) procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University were used in this study. The animals were fed on a standard pellet diet (Pranav Agro Industries, Pune, India) and water was freely available. The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% carbohydrates. It provided a metabolizable energy of 3600 kcal. The animals were maintained in a controlled environment (12-h light/dark cycle) and temperature (30 ± 2°C). All the experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. The experimental protocol was approved by the Animal Ethical Committee of Annamalai University (Reg. No. 160/99/CPCSEA; vide No. 169, 2003). The animals were acclimatized to the laboratory conditions before starting the experiment.

Chemicals

Rutin hydrate and streptozotocin were purchased from Sigma Chemical Co. (St Louis, MO). Ferric chloride, cholesterol, isopropyl alcohol, sodium meta periodate, triethanolamine, diphenyl carbazide, palmitic acid, dextran sulfate, digitonin, hydroxylamine hydrochloride, perchloric acid, heparin, glycerol trioleate, orcinol, galactose, mannose, trisodium phosphate, potassium tetraborate, p-dimethyl amino benzaldehyde, galactosamine, periodic acid, sodium meta arsenate, thiobarbituric acid, sialic acid and cysteine hydrochloride were purchased from S.D. Fine Chemicals (Mumbai, India). All the other chemicals and biochemicals used were of analytical grade.

Induction of experimental diabetes

Streptozotocin was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice before use. The overnight fasted rats were made diabetic with a single intraperitoneal injection of streptozotocin (50 mg kg^{-1}). Control rats were injected with citrate buffer alone. Diabetes was confirmed in the streptozotocin-injected rats by measuring the fasting plasma glucose levels 72-h post injection. After an overnight fast, blood was withdrawn by sinocular puncture (0.2 mL) from rats in tubes containing potassium oxalate and sodium fluoride as anticoagulant. Plasma was separated after centrifugation and glucose was estimated using a commercial glucose kit (Qualigens Diagnostics (Product No. 72101), Mumbai, India). Rats with plasma glucose levels above 13.89 mmol L^{-1} (250 mg dL^{-1}) were considered as diabetic (Kamalakkannan & Stanely Mainzen Prince 2006a) and were used in the experiment. Treatment with rutin was started on the third day after streptozotocin injection i.e. after the estimation of plasma glucose.

Experimental design

A pilot study was conducted with three different doses of rutin (25, 50 and 100 mg kg^{-1}) to determine the dose-dependent effect in streptozotocin-induced diabetic rats. It was found that 25, 50 and 100 mg kg^{-1} rutin significantly ($P < 0.05$) decreased plasma glucose levels, and 100 mg kg^{-1} rutin was more effective in reducing plasma glucose levels significantly ($P < 0.05$) after 45 days of experimental study. Hence, 100 mg kg^{-1} rutin was chosen for further study (Kamalakkannan & Stanely Mainzen Prince 2006a). The experimental approach was pharmacological and the doses of rutin used in this study were not easily reached in a standard diet.

Four groups of rats, each containing eight rats, were maintained for the study. Group 1, normal control; group 2, normal + rutin (100 mg kg^{-1}) (Kamalakkannan & Stanely Mainzen Prince 2006a); group 3, diabetic control; group 4, diabetic + rutin (100 mg kg^{-1}) (Kamalakkannan & Stanely Mainzen Prince 2006a). Rutin was suspended in carboxy

methyl cellulose (0.01 g mL^{-1}) and was orally administered to rats using an intragastric tube for 45 days. Normal control and diabetic control rats received Carboxy methyl cellulose alone.

After the last treatment all the rats were fasted overnight and killed by cervical decapitation. Blood was collected in tubes containing Ethyrene diamine tetraacetic acid (EDTA) as an anticoagulant. Plasma was obtained after centrifugation and used for various biochemical estimations. Liver, kidney, heart and brain tissues were excised immediately from the animals and stored at −20°C until used.

Extraction of lipids

Lipids were extracted from plasma and tissues by the method of Folch et al (1957) using a chloroform–methanol mixture (2:1, v/v). The tissues were rinsed thoroughly in cold physiological saline and dried by pressing between the folds of filter paper. The samples were homogenized in cold chloroform–methanol (2:1, v/v) and contents were extracted after 24 h. The extraction was repeated four times. The combined filtrate was washed with 0.7% KCl and the aqueous layer was discarded. The organic layer was made up to a known volume with chloroform and used for various estimations.

Estimation of cholesterol

The cholesterol content was estimated by the method of Zlatkis et al (1953). A sample of lipid extract (0.1 mL) was evaporated to dryness and 5.0 mL of ferric chloride–acetic acid reagent was added. Concentrated sulfuric acid (3.0 mL) was also added and after 20 min the absorbance was read at 560 nm.

Estimation of triglycerides

Triglycerides were estimated by the method of Foster & Dunn (1973). A sample of lipid extract was evaporated to dryness. Methanol (0.1 mL) and isopropyl alcohol (4.0 mL) were added. Alumina 0.4 mg was added to all the tubes, which were well-shaken for 15 min and centrifuged. Samples (2.0 mL) of the supernatant fluid was transferred to labelled tubes. The tubes were placed in a water bath at 65°C for 15 min for saponification after adding 0.6 mL of the saponification reagent followed by 0.5 mL acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420 nm.

Estimation of free fatty acids

Free fatty acids were estimated by the method of Falholt et al (1973). A 0.1-mL sample of lipid extract was evaporated to dryness. Phosphate buffer (1.0 mL; pH 6.4), 6.0 mL extraction solvent (chloroform–heptane–methanol, 5:5:1) and 2.5 mL copper reagent were added. All the tubes were shaken vigorously. Activated silicic acid (200 mg) was added and left aside for 30 min. The tubes were centrifuged and 3.0 mL of the copper layer was transferred to another tube containing 0.5 mL diphenyl carbazide and mixed carefully. The absorbance was read at 550 nm immediately.

Estimation of phospholipids

Phospholipid content was estimated by the method of Zilversmit & Davis (1950). To 0.1 mL lipid extract, was added 1.0 mL conc. sulfuric acid, 1.0 mL conc. nitric acid and digested to a colourless solution. To this, 1.0 mL 2.5% ammonium molybdate and 0.1 mL amino-2 naphthol 4-sulfonic acid (ANSA) were added. The volume was then made up to 5.0 mL with distilled water and the absorbance was read at 660 nm.

Estimation of lipoproteins

Cholesterol in the lipoprotein fractions was determined by the method of Zlatkis et al (1953). HDL cholesterol was analysed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg^{2+}. Low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated as follows:

$$\text{VLDL-C} = \text{triglycerides}/5 \quad (1)$$

$$\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C} + \text{VLDL-C}) \quad (2)$$

Assay of HMG CoA reductase activity

The ratio between HMG CoA and mevalonate in the tissues was taken as the index of the activity of HMG CoA reductase as described by the method of Rao & Ramakrishnan (1975). Equal volumes of fresh 10% tissue homogenate and diluted perchloric acid were mixed, kept for 5 min and centrifuged at 2000 *g* for 10 min. To 1.0 mL filtrate, 0.5 mL freshly prepared hydroxylamine reagent (alkaline hydroxylamine in the case of HMG CoA) was added and mixed. After 5 min, 1.5 mL ferric chloride was added and shaken well. Readings were taken after 10 min at 540 nm against a similarly-treated saline–arsenate blank. The ratio of HMG CoA to mevalonate was calculated. A lower ratio indicated higher enzyme activity and a higher ratio indicated lower enzyme activity.

Assay of lipoprotein lipase (LPL)

Lipoprotein lipase activity was assayed in plasma by the method of Korn (1955). The incubation mixture contained 0.3 mL ammonium chloride–ammonia buffer, 0.1 mL calcium chloride, 0.4 mL substrate and 0.2 mL plasma. The contents were incubated at 37°C for 60 min. At the end of the incubation period, 1.0 mL cold 0.2 M H_2SO_4 was added to arrest the reaction. In the case of control, plasma was added after the addition of 1.0 mL 0.2 M H_2SO_4. The liberated glycerol was estimated in a sample of the incubation mixture.

Assay of lecithin cholesterol acyltransferase (LCAT)

The activity of LCAT was assayed in plasma by the method of Hitz et al (1983).

Preparation of substrate

A pool of human plasma was heated at 56°C for 30 min to inactivate the LCAT. The inactivated plasma was incubated at 4°C for 15 min with 0.2% dextran sulfate, which produced an elimination of two-thirds of the lipoproteins (LDL + VLDL). This was sedimented by centrifugation at 1750 *g* for 15 min. The supernatant containing HDL was used as the substrate.

A sample of substrate (0.6 mL) was mixed with the enzyme (0.6 mL). A 0.2-mL sample of this mixture was mixed with 1.0 mL isopropyl alcohol while the remaining mixture was incubated at 27°C for 90 min. The precipitate was removed by centrifugation and the supernatant was taken for the estimation of free cholesterol (Zlatkis et al 1953). This represented the amount of free cholesterol present in the test sample at zero time.

After 90 min, a 0.2-mL sample of the incubated mixture was mixed with 1.0 mL isopropyl alcohol and the remaining mixture was incubated at 27°C for a further 90 min. At the end of 180 min, 0.2 mL of the incubated mixture was treated with 1.0 mL isopropyl alcohol to arrest the reaction. The precipitated protein in all the tubes were separated by centrifugation and the free cholesterol content in the supernatant was estimated (Zlatkis et al 1953).

Control tubes containing only the substrate were treated similarly to check for the complete inactivation of plasma during substrate preparations. LCAT activity was expressed as a function of the disappearance of free cholesterol during the incubation period.

Extraction of glycoproteins

The tissue samples were defatted before estimation. A weighed amount of defatted tissue was suspended in 3.0 mL 2 M HCl and heated at 90°C for 4 h. The sample was cooled and neutralized with 3.0 mL 2 M NaOH. Samples from this were used for estimation of hexose, hexosamine, sialic acid and fucose.

Estimation of hexose

Protein-bound hexoses were estimated by the method of Dubois & Gilles (1956). To 0.1 mL plasma or defatted tissue sample, 5.0 mL 95% ethanol was added, mixed and then centrifuged. The precipitate was dissolved in 1.0 mL 0.1 M NaOH. Subsequently, 1.0 mL distilled water and 1.0 mL of standards (20–100 μg) were set up along with the test. To all the tubes, 8.5 mL orcinol-sulfuric acid reagent was added and kept in a water bath for exactly 15 min at 90°C. The tubes were cooled in tap water and the colour developed was read at 540 nm against a blank.

Estimation of hexosamine

Protein bound hexosamine was estimated by the method of Wagner (1979). To 1.0 mL plasma or defatted tissue sample, 2.5 mL 3 M HCl was added and kept for 6 h in a boiling water bath, and then neutralized with 6 M NaOH. To 0.8 mL of the neutralized sample was added 0.6 mL acetyl acetone reagent. The tubes were heated in a boiling water bath for 30 min. After cooling, 2.0 mL Ehrlich's reagent was added and mixed well. Blank contained 0.8 mL water. Blank and standards were processed similarly. The colour developed was read at 540 nm.

Estimation of sialic acid

Sialic acid in plasma and tissues were estimated by the method of Warren (1959). To 0.2 mL plasma or defatted tissue sample, 0.5 mL water and 0.25 mL periodic acid were added and incubated at 37°C for 30 min. To this were added 0.2 mL sodium meta arsenate and 2.0 mL thiobarbituric acid and heated in a boiling water bath for exactly 6 min, cooled, and 5.0 mL acidified butanol was added. The absorbance was read at 540 nm against a reagent blank.

Estimation of fucose

Fucose was estimated by the method of Dische & Shettles (1948). Two tubes each containing 0.1 mL sample (labelled as control and test) were taken. To this was added 5.0 mL 95% ethanol, mixed well and then centrifuged. The precipitate was dissolved in 1.0 mL 0.1 M NaOH. Distilled water (1.0 mL) served as the blank. A series of standards in 1.0 mL volume were also set up along with the test. All the tubes were kept in ice-cold conditions and 4.5 mL H_2SO_4–water mixture was added. The tubes were kept in a boiling water bath for 3 min and cooled. Cysteine reagent (0.1 mL) was added to all the tubes, except control, and kept for 60 min at room temperature. The colour developed was read at 396 nm and 430 nm against the blank. The fucose content was calculated from the differences in the readings obtained at 396 nm and 430 nm, and then subtracting the values obtained without cysteine.

Estimation of protein

Protein content in the tissue homogenate was determined by the method of Lowry et al (1951). Tissue homogenate (0.5 mL) was precipitated with 0.5 mL 10% TCA, centrifuged for 10 min and the precipitate was dissolved in 1.0 mL 0.1 M sodium hydroxide. A 0.1-mL sample was taken and made up to 1.0 mL with distilled water. To this was added 4.5 mL alkaline copper reagent, and allowed to stand at room temperature for 10 min. After incubation, 0.5 mL Folin's-Ciocalteau reagent was added and after 20 min the blue colour developed was read at 620 nm. A standard curve was obtained using BSA. The protein content was expressed as mg (g tissue)$^{-1}$.

Statistical analysis

All the grouped data were analysed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) using SPSS software package, version 9.05. P values < 0.05 were considered as significant.

Results

Fasting plasma glucose levels were significantly ($P < 0.05$) increased in diabetic control rats (21.17 ± 1.63 mmol L^{-1}) when compared with normal control rats. Diabetic rats when treated with rutin significantly ($P < 0.05$) decreased the plasma glucose levels (7.89 ± 0.60 mmol L^{-1}) on comparison with diabetic control rats. Normal rats treated with rutin did not show any

significant ($P<0.05$) effect on plasma glucose levels (3.89 ± 0.30 mmol L^{-1}). The levels of plasma glucose in normal control rats was found to be 3.83 ± 0.29 mmol L^{-1}. A significant ($P<0.05$) decrease in plasma insulin levels was observed in diabetic control rats (6.89 ± 0.22 μU mL^{-1}) when compared with normal control rats and on treatment with rutin, the levels significantly ($P<0.05$) increased (10.92 ± 0.48 μU mL^{-1}) when compared with diabetic control rats. There was no significant change in the levels of plasma insulin in normal rats treated with rutin (13.74 ± 1.05 μU mL^{-1}). Plasma insulin levels in normal control rats were observed to be 13.67 ± 1.04 μU mL^{-1}.

Table 1 shows the levels of lipids and the activity of LPL and LCAT in plasma of normal and diabetic rats. Significantly ($P<0.05$) increased levels of cholesterol, triglycerides, free fatty acids and phospholipids, and significantly ($P<0.05$) decreased activity of LPL and LCAT were observed in plasma of diabetic control rats when compared with normal control rats. In rutin-treated diabetic rats, the lipid levels were significantly ($P<0.05$) decreased and the activity of LPL and LCAT was increased significantly ($P<0.05$) in the plasma when compared with diabetic control rats.

The levels of plasma LDL- and VLDL-cholesterol increased, whereas HDL-cholesterol decreased significantly ($P<0.05$) in diabetic control rats when compared with normal control rats. In rutin-treated diabetic rats, the levels of HDL-cholesterol increased and LDL- and VLDL-cholesterol levels decreased significantly ($P<0.05$) when compared with diabetic control rats (Table 2).

The levels of lipids in the liver and kidney are presented in Table 3, whilst the levels of lipids in the heart and brain are presented in Table 4. A significant ($P<0.05$) increase in the levels of lipids was observed in these tissues in diabetic control rats when compared with normal control rats. Oral administration of rutin to diabetic rats significantly ($P<0.05$) decreased the levels of lipids when compared with diabetic control rats.

A significant ($P<0.05$) increase in the activity of HMG CoA reductase was observed in the liver, kidney and heart of the diabetic control rats when compared with normal control rats. Oral administration of rutin to diabetic rats significantly ($P<0.05$) decreased the activity of HMG CoA reductase in these tissues on comparison with diabetic control rats (Table 5).

Table 6 represents the levels of glycoproteins (hexose, hexosamine, fucose and sialic acid) in plasma and tissues (liver and kidney) of normal and diabetic rats. Significantly ($P<0.05$) higher levels of glycoproteins were observed in plasma and tissues of diabetic control rats when compared with normal control rats. Treatment with rutin to diabetic rats resulted in a significant ($P<0.05$) reduction of glycoproteins in plasma and tissues when compared with diabetic control rats.

Discussion

Streptozotocin-induced diabetic rats exhibited higher levels of cholesterol, triglycerides, free fatty acids and phospholipids in plasma and tissues in this study. The rise in plasma lipids indicated increased mobilization of free fatty acids from peripheral depots. On the other hand, insulin inhibited the hormone sensitive lipase (Goodman & Gilman 1985). A

Table 1 Effect of rutin on plasma lipids and on the activity of plasma lipoprotein lipase (LPL) and lecithin cholesterol acyltransferase (LCAT) in normal and diabetic rats

Groups	Cholesterol	Triglycerides	Free fatty acids	Phospholipids	LPL (μmol glycerol liberated h^{-1} L^{-1})	LCAT (μmol cholesterol esterified h^{-1} L^{-1})
	(mg dL^{-1})					
Normal control	83.01 ± 6.32^{a}	21.56 ± 1.64^{a}	59.44 ± 4.53^{a}	101.57 ± 7.73^{a}	7.21 ± 0.55^{a}	69.24 ± 5.27^{a}
Normal + rutin (100 mg kg^{-1})	80.21 ± 6.11^{a}	20.23 ± 1.54^{a}	59.21 ± 4.51^{a}	99.02 ± 7.54^{a}	7.16 ± 0.61^{a}	69.15 ± 5.08^{a}
Diabetic control	214.54 ± 16.34^{b}	33.61 ± 2.56^{b}	158.53 ± 12.07^{b}	210.19 ± 16.00^{b}	5.08 ± 0.39^{b}	58.61 ± 4.46^{b}
Diabetic + rutin (100 mg kg^{-1})	110.50 ± 8.42^{c}	25.86 ± 1.97^{c}	78.67 ± 5.99^{c}	133.14 ± 10.14^{c}	6.41 ± 0.49^{c}	64.34 ± 4.90^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly from each other ($P<0.05$, DMRT).

Table 2 Effect of rutin on plasma lipoproteins in normal and diabetic rats

Groups	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol
	(mg dL^{-1})		
Normal control	22.64 ± 1.72^{a}	56.06 ± 4.27^{a}	4.31 ± 0.33^{a}
Normal + rutin (100 mg kg^{-1})	23.08 ± 1.76^{a}	53.08 ± 4.04^{a}	4.05 ± 0.31^{a}
Diabetic control	16.33 ± 1.24^{b}	191.52 ± 14.58^{b}	6.72 ± 0.51^{b}
Diabetic + rutin (100 mg kg^{-1})	20.40 ± 1.55^{c}	84.94 ± 6.47^{c}	5.17 ± 0.39^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly from each other ($P<0.05$, DMRT).

Table 3 Effect of rutin on the concentrations of cholesterol, triglycerides, free fatty acids and phospholipids in the liver and kidney of normal and diabetic rats

	Liver (mg (g tissue)$^{-1}$)				**Kidney (mg (g tissue)$^{-1}$)**			
Groups	Cholesterol	Triglycerides	Free fatty acids	Phospholipids	Cholesterol	Triglycerides	Free fatty acids	Phospholipids
Normal control	5.31 ± 0.40^{a}	3.93 ± 0.30^{a}	7.25 ± 0.55^{a}	20.40 ± 1.55^{a}	3.12 ± 0.24^{a}	2.33 ± 0.18^{a}	6.63 ± 0.50^{a}	17.23 ± 1.31^{a}
Normal + rutin (100 mg kg^{-1})	5.06 ± 0.39^{a}	3.54 ± 0.27^{a}	6.90 ± 0.53^{a}	19.88 ± 1.51^{a}	2.94 ± 0.22^{a}	2.10 ± 0.16^{a}	6.20 ± 0.47^{a}	16.85 ± 1.28^{a}
Diabetic control	11.02 ± 0.84^{b}	9.40 ± 0.72^{b}	15.00 ± 1.14^{b}	39.52 ± 3.01^{b}	7.33 ± 0.56^{b}	5.84 ± 0.44^{b}	11.29 ± 0.86^{b}	30.60 ± 2.33^{b}
Diabetic + rutin (100 mg kg^{-1})	7.93 ± 0.60^{c}	6.81 ± 0.52^{c}	9.89 ± 0.75^{c}	25.00 ± 1.90^{c}	4.80 ± 0.37^{c}	3.95 ± 0.30^{c}	8.33 ± 0.63^{c}	21.90 ± 1.67^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT).

Table 4 Effect of rutin on the concentrations of cholesterol, triglycerides, free fatty acids and phospholipids in the heart and brain of normal and diabetic rats

Groups	Heart (mg g tissue)$^{-1}$)				Brain (mg (g tissue)$^{-1}$)			
	Cholesterol	Triglycerides	Free fatty acids	Phospholipids	Cholesterol	Triglycerides	Free fatty acids	Phospholipids
Normal control	2.81 ± 0.21^{a}	3.24 ± 0.25^{a}	3.77 ± 0.29^{a}	6.08 ± 0.46^{a}	8.60 ± 0.65^{a}	3.08 ± 0.23^{a}	1.65 ± 0.13^{a}	21.20 ± 1.61^{a}
Normal + rutin (100 mg kg^{-1})	2.54 ± 0.19^{a}	3.10 ± 0.24^{a}	3.61 ± 0.27^{a}	5.82 ± 0.44^{a}	8.51 ± 0.65^{a}	2.90 ± 0.22^{a}	1.53 ± 0.12^{a}	20.54 ± 1.56^{a}
Diabetic control	4.80 ± 0.37^{b}	5.34 ± 0.41^{b}	6.50 ± 0.50^{b}	10.23 ± 0.78^{b}	17.42 ± 1.33^{b}	5.86 ± 0.45^{b}	3.88 ± 0.30^{b}	32.12 ± 2.45^{b}
Diabetic + rutin (100 mg kg^{-1})	3.44 ± 0.26^{c}	4.19 ± 0.32^{c}	4.58 ± 0.35^{c}	7.71 ± 0.59^{c}	10.65 ± 0.81^{c}	3.77 ± 0.29^{c}	2.15 ± 0.16^{c}	25.08 ± 1.91^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT).

Table 5 Effect of rutin on the activity of HMG CoA reductase in the liver, kidney and heart of normal and diabetic rats

Groups	Liver	Kidney	Heart
	(HMG CoA/mevalonate ratio*)		
Normal control	1.62 ± 0.06^{a}	1.38 ± 0.05^{a}	0.80 ± 0.03^{a}
Normal + rutin (100 mg kg^{-1})	1.67 ± 0.06^{a}	1.41 ± 0.04^{a}	0.86 ± 0.03^{a}
Diabetic control	0.88 ± 0.08^{b}	0.79 ± 0.06^{b}	0.42 ± 0.03^{b}
Diabetic + rutin (100 mg kg^{-1})	1.34 ± 0.09^{c}	1.16 ± 0.08^{c}	0.65 ± 0.04^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT). *Lower ratio indicates higher enzyme activity and higher values indicate lower enzyme activity.

deficiency of insulin is associated with an increase in cholesterol levels due to the enhanced mobilizations of lipids from the adipose tissue to the plasma (Rajalingam et al 1987). An increase in cholesterol levels in the hepatic tissue might be due to an increase in the transport of chylomicron cholesterol to the liver (Chauhan et al 1987). Hypertriglyceridaemia in diabetes can result from an increased hepatic VLDL overproduction and impaired catabolism of triglyceride-rich particles. Dysfunction of LPL also contributes to hypertriglyceridaemia in the fasting and postprandial state (Niemeijer-Kanters et al 2001).

In insulin-deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis (Ghosh & Suryawanshi 2001). During diabetes, the heart is under stress and therefore may preferentially utilize glucose for its energy production, resulting in a rise in the concentration of free fatty acid in the myocardium (Parving & Hommel 1989). The higher levels of phospholipid in diabetic liver is due to increased hepatic synthesis. It has been reported that increased incorporation of ^{32}Pi into plasma phosphatidyl choline in diabetic rats is responsible for increased hepatic synthesis (Chauhan et al 1987). An increase in phospholipid levels in the heart could be due to an increased phospholipid content of its membrane.

The normal metabolism of brain is altered in the diabetic state. Increased cholesterol levels in diabetic brain may be associated with a relatively large increase in molecular ordering of residual phospholipid resulting in a decrease in membrane fluidity, rendering lipid dependent membrane bound enzymes non-functional (Makar et al 1995). The brain can extract fatty acids from the plasma and this may be responsible for the higher levels of free fatty acids in diabetic brain (Yagi 1987).

Decreased levels of cholesterol, triglycerides, free fatty acids and phospholipids were found in plasma and tissues of rutin-treated diabetic rats. This could have been due to an increase in the levels of insulin by the administration of rutin. In this context, we have reported previously that rutin administration to streptozotocin-induced diabetic rats for 45 days increased the levels of plasma insulin. Rutin, by its ability to scavenge free radicals and to inhibit lipid peroxidation, prevented streptozotocin-induced oxidative stress and protected beta cells resulting in increased insulin secretion and decreased blood glucose levels (Kamalakkannan & Stanely Mainzen Prince 2006a). In this context, Coskun et al (2005) have reported that, in streptozotocin-induced diabetic rats, quercetin protected pancreatic beta cells by decreasing oxidative stress and preserving beta cell integrity. Insulin profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase. Insulin inhibits the activity of the lipase primarily through reductions in cAMP levels, owing to the activation of a cAMP-specific phosphodiesterase in fat cells (Botion & Green 1999). Rutin, one of the constituents of hawthorn fruit, decreased serum total cholesterol (Weihmayr & Ernst 1996), LDL-cholesterol and triglycerides in hyperlipidaemic patients (Chen et al 1995).

Decreased activity of LCAT was observed in the plasma of diabetic rats in this study. The decreased activity of LCAT indicated impairment in HDL-cholesterol synthesis as well as triglyceride metabolism in diabetic rats. Decreased LPL activity was observed in this study. A decrease in plasma LPL activity was reported in streptozotocin-induced diabetes mellitus (Tsutsumi et al 1995). The decreased activity of LPL in experimental animals and diabetic patients results from insulin-deficiency, since its synthesis is induced by insulin (Garfinkel et al 1976). The activity of HMG CoA reductase increased in diabetic rats. A deficiency of insulin is associated with an increase in HMG CoA reductase activity (Catanzaro & Suen 1996).

The lipoprotein profile in diabetes is abnormal, with hypertriglyceridaemia and reduced HDL-cholesterol plasma concentrations as the dominant features. Increased VLDL and LDL-cholesterol levels with a decrease in the levels of HDL-cholesterol were observed in the plasma of diabetic rats. The breakdown of VLDL led to the formation of the cholesterol-rich particle LDL (Catanzaro & Suen 1996). Oxidation of lipoproteins is a characteristic event in the oxidative stress caused by the oxidative damage to polyunsaturated fatty acids (Korytar et al 2002). Increased glycation of LDL also occurs in diabetes. Glycated LDL is not recognized by the LDL receptor but its uptake by macrophages is enhanced (Lopes-Virella et al 1988) and this may account, at least in part, for the hyperlipidaemia and accelerated foam cell formation in diabetes. HDL is involved in the transport of cholesterol from peripheral tissues into the liver and it acts as a protective factor against coronary heart disease (Gordon et al 1977).

Rutin when administered orally to diabetic rats significantly reduced the levels of LDL and VLDL-cholesterol and increased that of HDL-cholesterol. The flavonoids appeared to act by protecting LDL against oxidation caused by the macrophages, as they inhibited the generation of hydroperoxides and protected α-tocopherol carried in lipoproteins from being consumed by oxidation in the LDL (Middleton et al 2000). The facilitation of atherogenesis by LDL is due to its role in depositing cholesterol in the vascular bed.

Table 6 Effect of rutin on the levels of glycoproteins in plasma, liver and kidney of normal and diabetic rats

Groups	Plasma (mg dL^{-1})				Liver (mg (g defatted tissue)$^{-1}$)				Kidney (mg (g defatted tissue)$^{-1}$)			
	Hexose	Hexosamine	Fucose	Sialic acid	Hexose	Hexosamine	Fucose	Sialic acid	Hexose	Hexosamine	Fucose	Sialic acid
Normal control	86.81 ± 6.61^{a}	67.51 ± 5.14^{a}	36.43 ± 2.77^{a}	62.55 ± 4.76^{a}	33.02 ± 2.55^{a}	18.65 ± 1.42^{a}	12.54 ± 0.88^{a}	8.32 ± 0.63^{a}	28.51 ± 2.17^{a}	21.25 ± 1.57^{a}	13.80 ± 0.99^{a}	7.55 ± 0.58^{a}
Normal + rutin (100 mg kg^{-1})	85.01 ± 6.70^{a}	66.01 ± 5.03^{a}	36.01 ± 2.70^{a}	62.17 ± 4.61^{a}	32.59 ± 2.58^{a}	18.02 ± 1.37^{a}	12.32 ± 0.84^{a}	7.60 ± 0.62^{a}	28.14 ± 2.20^{a}	20.86 ± 1.59^{a}	13.22 ± 1.01^{a}	7.08 ± 0.54^{a}
Diabetic control	129.02 ± 9.82^{b}	92.42 ± 7.04^{b}	48.61 ± 3.70^{b}	84.64 ± 6.45^{b}	52.54 ± 4.31^{b}	30.22 ± 2.23^{b}	20.30 ± 1.70^{b}	14.50 ± 1.33^{b}	50.08 ± 3.96^{b}	32.44 ± 2.17^{b}	25.40 ± 2.24^{b}	13.55 ± 1.03^{b}
Diabetic + rutin (100 mg kg^{-1})	94.02 ± 7.77^{c}	76.01 ± 5.79^{c}	39.33 ± 3.15^{c}	68.03 ± 5.18^{c}	38.03 ± 3.12^{c}	22.65 ± 1.72^{c}	15.80 ± 1.20^{c}	10.20 ± 0.85^{c}	35.67 ± 2.94^{c}	24.00 ± 1.75^{c}	18.23 ± 1.39^{c}	9.88 ± 0.75^{c}

Each value is mean ± s.d. for eight rats in each group. Values that have a different superscript letter (a, b, c) differ significantly with each other ($P < 0.05$, DMRT).

HDL-cholesterol, however carries out the reverse transport of excess cholesterol from cells of tissues to the liver or to cells whose cholesterol requirements exceed their capacity for synthesis (Zilva & Pannall 1975). Rutin thus has the potential to prevent the formation of atherosclerosis and coronary heart disease, which are the secondary complications of diabetes mellitus.

Treatment of diabetic rats with rutin had a positive effect on the marker enzymes. The activity of HMG CoA reductase decreased and that of LPL and LCAT increased in rutin-treated diabetic rats. Insulin deficiency is associated with an increase in HMG CoA reductase activity and a decrease in LPL activity in the diabetic state, and so increased insulin levels in the diabetic rats administered with rutin decreased the activity of HMG CoA reductase and increased LPL activity. Restoration of the lipid profile in diabetic rats could have been the cause of increased LCAT activity. In this context, a number of bioflavonoids such as epicatechin, quercetin and hesperidin were shown to possess lipid lowering properties (Basarkar & Nath 1981).

Increased glycosylation of various proteins in diabetic patients has been reported (Rahman et al 1990). In this study, we have observed increased levels of hexose, hexosamine, fucose and sialic acid in plasma and tissues of streptozotocin-treated diabetic rats. The increase in plasma glycoprotein components has been associated with the severity and duration of diabetes. In hyperglycaemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff's-base intermediate (Maillard reaction). These Schiff-base intermediates undergo Amadori rearrangement to stable ketoamine derivative (fructosamine) (Bucala 1999).

Fucose is a member of a group of eight essential sugars that 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 (Mondoa et al 2001). A raise in fucose levels could be due to increased glycosylation in the diabetic state. Sialic acid is found in a wide variety of substances and tissues in animals and man, occurring most abundantly in glycoproteins and glycolipids. Sialic acid bound to membrane glycoproteins and glycolipids apparently enters the circulation by either shedding or cell lysis (Sheshadri 1994). Increased levels of sialic acid were reported in streptozotocin-induced diabetic rats (Gorgun et al 2002).

In the diabetic state, deficiency of insulin secretion causes derangement of glycoprotein metabolism, and this results in the basal membrane thickening. Excess availability of glucose in the hyperglycaemic state accelerates the synthesis of basement membrane components i.e. glycoproteins (Spiro & Spiro 1971). Rutin administration to diabetic rats decreased the levels of glycoproteins in plasma and tissues. A decreased hyperglycaemic state with increased levels of plasma insulin observed in rutin-treated diabetic rats might have been responsible for the decrease of glycoproteins in plasma, liver and kidney. In this context, other researchers have shown that a decrease in hyperglycaemia could lead to a decrease in glycoprotein levels (Latha & Pari 2005).

Agents with antioxidant or free radical scavenging properties may inhibit oxidative reactions associated with glycation (Asgary et al 1999). Rutin is an antioxidant and is able to quench the free radicals responsible for the increased oxidative stress in diabetic rats. Further, rutin was shown to inhibit glycosylation reactions by reducing the generation of reactive oxygen species (Asgary et al 1999). Previously, we reported that rutin decreased glycosylated haemoglobin levels, decreased lipid peroxidation and improved antioxidant status in plasma and tissues of streptozotocin-diabetic rats (Kamalakkannan & Stanely Mainzen Prince 2006a,c).

References

Asgary, S., Naderi, G., Sarrafzadgen, N., Ghassemi, N., Boshtam, M., Rafie, M., Arefian, A. (1999) Antioxidant effect of flavonoids on hemoglobin glycosylation. *Pharm. Acta Helv*. **73**: 223–236

Basarkar, P. W., Nath, N. (1981) Cholesterol lowering action of vitamin P-like compounds in rats. *Indian J. Exp. Biol*. **19**: 787–789

Baynes, J. W. (1991) Role of oxidative stress in development of complications of diabetes mellitus. *Diabetes* **40**: 405–412

Botion, L. M., Green, A. (1999) Long-term regulation of lipolysis and hormone-sensitive lipase by insulin and glucose. *Diabetes* **48**: 1691–1697

Bucala, R. (1999) Advanced glycosylation end products and diabetic vascular disease. In: Keaney, J. F. Jr. (ed.) *Oxidative stress and vascular disease*. Kluwer Academic Publishers, Dordrecht, pp. 287–303

Catanzaro, N. D. J. A., Suen, M. T. R. (1996) Clinical laboratory indicators of cardiovascular disease risk. *Altern. Med. Rev*. **1**: 185–194

Chauhan, U. P. S., Jagi, C. B., Singh, V. N. (1987) Incorporation of ^{32}Pi into plasma phosphatidylcholine of diabetic rats. *Indian J. Nucl. Med*. **2**: 92–98

Chen, J. D., Wu, Y. Z., Tao, Z. L., Chen, Z. M., Liu, X. P. (1995) Hawthorn (shan zha) drink and its lowering effect on blood lipid levels in humans and rats. *World Rev. Nutr. Diet* **77**: 147–154

Coskun, O., Kanter, M., Korkmaz, A., Oter, S. (2005) Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. *Pharmacol. Res*. **51**: 117–123

Dische, L., Shettles, L. B. (1948) Specific colour reactions of methyl pentoses and spectrophotometric micromethod for their determination. *J. Biol. Chem*. **175**: 595–604

Dubois, M., Gilles, K. A. (1956) In: *Methods in enzymology. Academic Press*, New York. p. 83

Falholt, K., Falholt, W., Lund, B. (1973) An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin. Chim. Acta* **46**: 105–111

Folch, J., Lees, M., Solane, S. G. H. (1957) A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **26**: 487–509

Foster, L. V., Dunn, R. T. (1973) Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. *Clin. Chem*. **19**: 338–340

Garber, A. J. (2002) Attenuating CV risk factors in patients with diabetes: clinical evidence to clinical practice. *Diab. Obes. Metab*. **4**: S5–S12

Garfinkel, A. G., Nilsson-ehle, P., Schotz, M. C. (1976) Regulation of lipoprotein lipase. Induction by insulin. *Biochim. Biophys. Acta* **424**: 264–273

Ghosh, S., Suryawanshi, S. A. (2001) Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J. Exp. Biol*. **39**: 748–759

Goodman, L.S., Gilman, A. (1985) In: *The Pharmacological basis of therapeutics*. McMillan, New York, pp 1490–1510

Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., Dawber, T. R. (1977) Diabetes, blood lipids, and the role of obesity in coronary heart disease risk for women. The Framingham study. *Ann. Intern. Med*. **87**: 393–397

Gorgun, F. M., Oztruk, Z., Gumustas, K. M., Kokoglu, E. (2002) Melatonin administration affects plasma total sialic acid and lipid peroxidation levels in streptozotocin induced diabetic rats. *J. Toxicol. Environ. Health Part A* **65**: 695–700

Havsteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol*. **32**: 1141–1148

Hertog, M. G., Hollman, P. C., Katan, M. B., Kromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **20**: 21–29

Hitz, J., Steinmetz, J., Siest, G. (1983) Plasma lecithin:cholesterol acyltransferase:reference values and effects of xenobiotics. *Clin. Chim. Acta* **133**: 85–96

Kamalakkannan, N., Stanely Mainzen Prince, P. (2006a) Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats. *Basic Clin. Pharmacol. Toxicol*. **98**: 97–103

Kamalakkannan, N., Stanely Mainzen Prince, P. (2006b) The influence of rutin on the extracellular matrix in streptozotocin induced diabetic rat kidney. *J. Pharm. Pharmacol*. **58**: 1091–1098

Kamalakkannan, N., Stanely Mainzen Prince, P. (2006c) Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. *Mol.Cell.Biochem*. In press

Karthick, M., Stanely Mainzen Prince, P. (2006) Preventive effect of rutin, a bioflavonoid, on lipid peroxides and antioxidants in isoproterenol induced myocardial infarction in rats. *J. Pharm. Pharmacol*. **58**: 701–707

Korn, E. D. (1955) Clearing factor: a heparin activated lipoprotein lipase: isolation and characterization of enzyme from normal rats. *J. Biol. Chem*. **215**: 1–26

Korytar, P., Molnarova, M., Sivonova, M., Zitnanova, I., Ulicna, O., Liptakova, A., Carsky, J., Durackova, Z. (2002) Effects of aminoguanidine Schiff's base on biomarkers of the oxidative stress, 4-hydroxy-2-nonenal and conjugated dienes, in the model diabetes mellitus. *Gen. Physiol. Biophys*. **21**: 337–344

Krishna K. M., Annapurna A, Gopal G. S., Chalam, C. R., Mandan, K., Kumar, V. K., Prakash, G. J. (2005) Partial reversal by rutin and quercetin of impaired cardiac function in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol*. **83**: 343–355

Latha, M., Pari, L. (2005) Effect of an aqueous extract of *Scoparia dulcis* on plasma and tissue glycoproteins in streptozotocin induced diabetic rats. *Pharmazie* **60**: 151–154

Lopes-Virella, M. F., Klein, R. L., Lyons, T. J., Stevenson, H. C., Witztum, J. L. (1988) Glycosylation of low-density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages. *Diabetes* **37**: 550–557

Lowry, O. H., Rosebrough, N. J., Farr, A .L, Randall, R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem*. **193**: 265–275

Makar, T. K., Hungund, B. L., Cook, G. A., Kashfi, K., Cooper, A. J. (1995) Lipid metabolism and membrane composition are altered in the brains of type II diabetic mice. *J. Neurochem*. **64**: 2159–2168

Middleton, E., Kandaswami, C., Theoharides, T. C. (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacol. Rev*. **52**: 673–751

Mondoa, E. I., Kitei, M. (2001) The new healing science of glyconutrients. In: *Sugars that heal*. Ballantine Publishing, New York

Niemeijer-Kanters, S. D. J. M., Banga, J. D., Erkelens, D. W. (2001) Lipid-lowering therapy in diabetes mellitus. *Neth. J. Med*. **58**: 214–222

Parving, H. H., Hommel, E. (1989) Prognosis in diabetic nephropathy. *Br. Med. J*. **299**: 230–237

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

Rajalingam, R., Srinivasan, N., Govindarajulu, P. (1987) Effect of alloxan induced diabetes on lipid profiles in renal cortex and medulla of mature albino rats. *Indian J. Exp. Biol*. **31**: 577–579

Rao, V., Ramakrishnan, S. (1975) Indirect assessment of hydroxy methyl glutaryl CoA reductase activity in liver tissue. *J. Clin. Chem*. **21**: 1523–1527

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

Sheshadri, N. (1994) Sialic acid as a tumor marker. *Ann. Clin. Lab. Sci*. **24**: 476–484

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

Stanely Mainzen Prince, P., Kamalakkannan, N. (2006) Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. *J. Biochem. Mol. Toxicol*. **20**: 96–102

Tsutsumi, K., Inoue, Y., Shima, A., Murase, T. (1995) Correction of hypertriglyceridemia with low high-density lipoprotein cholesterol by the novel compound NO-1886, a lipoprotein lipase-promoting agent, in STZ-induced diabetes rats. *Diabetes* **44**: 414–417

Wagner, W. D. (1979) A more sensitive assay discriminating galactosamine and glucosamine in mixture. *Anal. Biochem*. **94**: 394–396

Warren, L. (1959) Thiobarbituric acid assay of sialic acid. *J. Biol. Chem*. **234**: 1974–1975

Weihmayr, T., Ernst, E. (1996) Therapeutic effectiveness of Crataegus. *Fortschr Med*. **114**: 27–29

Yagi, K. (1987) Lipid peroxides and human diseases. *Chem. Phys. Lipids* **45**: 337–351

Zilva, J. F., Pannall, P. R. (1975) In: *Clinical chemistry in diagnosis and treatment*. 2nd edn, Lloyd-Luke, London, p. 8

Zilversmit, D. B., Davis, A .K. (1950) Microdetermination of plasma phospholipids by TCA precipitation. *J. Lab. Clin. Med*. **35**: 155–159

Zlatkis, A., Zak, B., Boyle, G. J. (1953) A method for the determination of serum cholesterol. *J. Clin. Med*. **41**: 486–492