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Antihyperglycaemic and Antioxidant Effect of Rutin, a Polyphenolic Flavonoid, in Streptozotocin-Induced Diabetic Wistar Rats

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Abstract: Flavonoids are non-nutritive dietary components that are widely distributed in plants. The present study investigated the antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid in normal and streptozotocin-induced diabetic Wistar rats. Diabetes as induced in rats by an intraperitoneal injection of streptozotocin. Rutin was orally administered to normal and diabetic rats for a period of 45 days. Fasting plasma glucose, glycosylated haemoglobin, thiobarbituric acid reactive substances and lipid hydroperoxides were significantly ($P<0.05$) increased, whereas insulin, C-peptide, total haemoglobin, protein levels, non-enzymic antioxidants (glutathione, vitamin C, vitamin E and ceruloplasmin) were decreased significantly ($P<0.05$) in diabetic rats. Oral administration of rutin to diabetic rats significantly ($P<0.05$) decreased fasting plasma glucose, glycosylated haemoglobin and increased insulin, C-peptide, haemoglobin and protein levels. Administration of rutin also decreased thiobarbituric acid reactive substances and lipid hydroperoxides and increased the non-enzymic antioxidants significantly ($P<0.05$). Treatment of normal rats with rutin did not significantly ($P<0.05$) alter any of the parameters studied. These results show that rutin exhibits antihyperglycaemic and antioxidant activity in streptozotocin-induced diabetic rats.

Diabetes mellitus is a serious metabolic disorder with micro- and macrovascular complications that results in significant morbidity and mortality. The increasing number of ageing populations, consumption of calorierich diets, obesity and sedentary life-style have lead to a tremendous increase in the number of patients with diabetes worldwide (Simpson *et al.* 2003). The World Health Organization (WHO) has predicted that the worldwide number of patients with diabetes will double by the year 2025, from the current number of approximately 150 million to 300 million (World Health Organization 2002). Recently, the search for appropriate hypoglycaemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatment than the currently used drugs (Rates 2001).

Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski 2001). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes *de novo* free radicals generation (Baynes & Thorpe 1997). Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of streptozotocin (Alvarez *et al.* 2004; Coskun *et al.* 2005).

Under physiological conditions, a wide range of antioxidant defences protects against the adverse effects of free radical production *in vivo* (Halliwell & Gutteridge 1990). In diabetes mellitus, protein glycation and glucose auto-oxidation may generate free radicals, which in turn catalyse lipid peroxidation (Baynes 1991). Disturbances of antioxidant defence systems in diabetes mellitus such as alteration in antioxidant enzymes (Maritim *et al.* 2003; Seif & Youssef 2004), lowered vitamin levels (West 2000), decreased ceruloplasmin levels (Anwar & Meki 2003; Seif & Youssef 2004) have been reported. Decreased lipid peroxidation and improved antioxidant status may be one mechanism by which dietary treatment contributes to the prevention of diabetic complications (Armstrong *et al.* 1996).

Flavonoids represent the most common and widely distributed group of plant phenolics (Harborne 1986) and are abundant in foods. Quercetin (3,3′,4′,5,7-pentahydroxy flavone) (fig. 1) is one of the most common native flavonoids occurring mainly in glycosidic forms such as rutin (5,7,3′,4′-OH, 3-rutinose) (fig. 2) (Havsteen 1983). Quercetin and rutin are the flavonoids most abundantly consumed in foods (Nakamura *et al.* 2000). Rutin is abundantly present in onions, apples, tea and red wine (Hertog *et al.* 1993). Rutin exhibits multiple pharmacological activities including antibacterial, antitumour, antiinflammatory, antidiarrhoeal, antiulcer, antimutagenic, myocardial protecting, vasodilator, immunomodulator and hepatoprotective activities (Janbaz *et al.* 2002). Much interest has gath-

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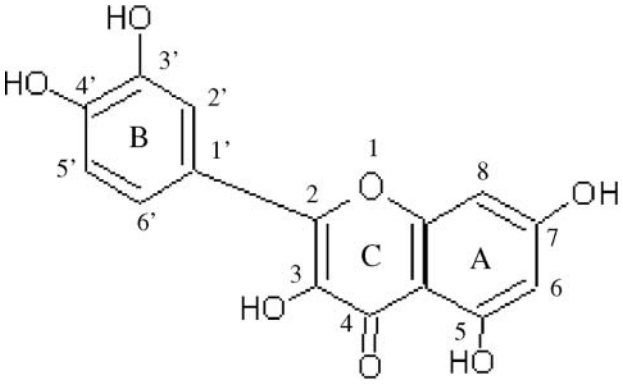

Fig. 1. Quercetin (3,3′,4′,5,7-pentahydroxy flavone).

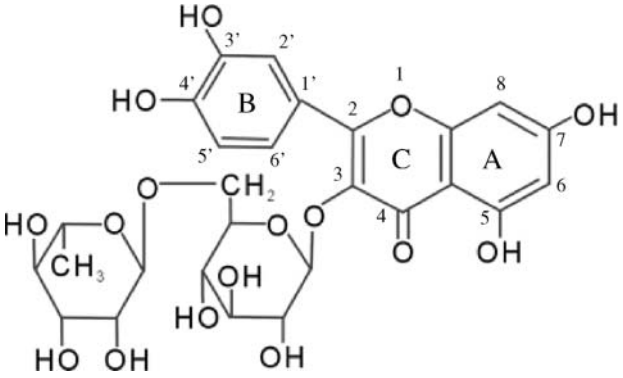

Fig. 2. Rutin (5,7,3′,4′-OH, 3-rutinose).

ered in the role and usage of natural antioxidants as a means to prevent oxidative damage in diabetes with high oxidative stress.

Hence, the present study was designed to investigate the effects of rutin, a polyphenolic flavonoid on blood glucose, insulin and C-peptide levels, total and glycosylated haemoglobin, plasma total proteins, thiobarbituric acid reactive substances, lipid hydroperoxides and plasma non-enzymic antioxidants (glutathione, vitamin C, vitamin E and ceruloplasmin) in normal and streptozotocin-induced experimental diabetic rats.

Materials and Methods

Experimental animals. Male albino Wistar rats (150–180 g) obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University were used in this study. The animals were fed a standard pellet diet (Pranav Agro Industries, Pune, India) and water was freely available. They were maintained in a controlled environment (12:12 hr light/dark cycle) and temperature (30±2°). The experimental protocol was approved by the Ethical Committee of Annamalai University.

Chemicals. Rutin hydrate and streptozotocin were purchased from Sigma Chemical Co., St. Louis, MO, USA. Fructose, phosphotungstic acid, thiobarbituric acid, 1,1′,3,3′ tetramethoxy propane, butylated hydroxy toluene, xylenol orange, dithionitro bis benzoic acid, ascorbic acid, 2,2′ dipyridyl, p-phenylene diamine hydrochloride and sodium azide were obtained from S.D. Fine Chemicals, Mumbai, India. All the chemicals used in the present study are of analytical grade.

Induction of experimental diabetes. Diabetes was induced in 12 hr fasted rats with streptozotocin (50 mg/kg) dissolved in citrate buffer (0.01 M, pH 4.5) intraperitoneally and the injection volume was 1 ml/rat. Control animals were injected with citrate buffer alone. After 72 hr of streptozotocin injection, blood was withdrawn from animals (sinocular puncture) fasted overnight in tubes containing potassium oxalate and sodium fluoride as anticoagulant and plasma glucose was estimated using a commercial glucose kit (Product No. 72101) provided by Qualigens Diagnostics, Mumbai, India. Rats that had a fasting plasma glucose value of above 13.89 mmol/l (250 mg/dl) were included in the study as diabetic rats (Cam *et al.* 2003).

Experimental design. The rats were divided into 8 groups of 8 rats in each group as follows: Group I: Normal control; Group II: Normal + rutin (25 mg/kg); Group III: Normal + rutin (50 mg/kg); Group IV: Normal + rutin (100 mg/kg); Group V: Diabetic control; Group VI: Diabetic + rutin (25 mg/kg); Group VII: Diabetic + rutin (50 mg/kg); and Group VIII: Diabetic + rutin (100 mg/kg). Rutin was suspended in carboxy methyl cellulose (CMC) (0.1 g %) and orally administered to rats (1 ml/rat) using an intragastric tube for a period of 45 days.

Blood samples were withdrawn from animals (sinocular puncture) on day 15, 30 and 45 and the fasting plasma glucose levels were estimated. After the last treatment, rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected, plasma and serum were obtained and used for various biochemical estimations.

Biochemical estimations. Plasma insulin and C-peptide were assayed by an enzyme linked immunosorbent assay (ELISA) method using commercial kits (Catalog No. SP-401 and LKPE1 respectively) from United Biotech Inc., Mountain View, CA, USA. Total haemoglobin and total proteins were estimated using commercial kits (Product No. 72091 and 72111 respectively) from Qualigens Diagnostics, Mumbai, India. Glycosylated haemoglobin (Bannon 1982), plasma thiobarbituric acid reactive substances (Yagi 1987) and plasma lipid hydroperoxides (HP) (Jiang *et al.* 1992) were estimated.

Estimation of reduced glutathione. Reduced glutathione (GSH) in plasma was estimated by the method of Ellman (1959). 0.5 ml of plasma was pipetted out and precipitated with 2.0 ml of 5% trichloroacetic acid. One ml of the supernatant was taken after centrifugation. To this, added 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The colour developed was read at 412 nm. A series of standards were treated in similar manner along with a blank containing 3.5 ml of buffer.

Estimation of vitamin C. Plasma vitamin C was estimated by the method of Omaye *et al.* (1979). 0.5 ml of plasma was mixed thoroughly with 1.5 ml of 6% trichloroacetic acid and centrifuged for 20 min. at 3,500×g. To 0.5 ml of the supernatant, 0.5 ml of 2,4 dinitro phenyl hydrazine (DNPH) reagent was added and mixed well. The tubes were allowed to stand at room temperature for 3 hr, removed and they were later placed in ice-cold water. To this, 2.5 ml of 85% sulphuric acid was added and allowed to stand for 30 min. A set of standards containing 10–50 µg of ascorbic acid were taken and processed similarly along with a blank containing 0.5 ml of 4% trichloroacetic acid. The colour developed was read at 530 nm.

Estimation of vitamin E. The levels of vitamin E in plasma was estimated by the method of Baker *et al.* (1951). To 0.1 ml of plasma, 1.5 ml of double distilled ethanol and 2.0 ml of petroleum ether (60–80°) were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80°. To this was added 0.2 ml of 0.2% 2,2′ dipyridyl solution and 0.2 ml of 0.5% ferric chloride solution.

Table 1.

Effect of rutin on body weight (g) in normal and diabetic rats. Each value is mean±S.D. for 8 rats in each group (n=8). Values that have a different superscript letter (a,b,c,d) differ significantly with each other (P<0.05, Duncan's multiple range test).

Groups	Initial	Final
Normal control	161.40±9.76	245.67±14.85[a]
Normal+rutin (25 mg/kg)	155.83±11.87	253.04±14.75[a]
Normal+rutin (50 mg/kg)	170.53±9.94	252.04±14.50[a]
Normal+rutin (100 mg/kg)	164.23±12.51	260.04±19.80[a]
Diabetic control	172.53±13.14	131.02±9.98[b]
Diabetic+rutin (25 mg/kg)	175.66±11.25	136.18±8.72[c]
Diabetic+rutin (50 mg/kg)	166.65±11.27	140.60±9.51[c]
Diabetic+rutin (100 mg/kg)	162.03±12.34	149.02±11.35[d]

Table 2.

Effect of rutin on food intake and water intake in normal and diabetic rats. Each value is mean±S.D. Values are given for each group that contain 8 rats (n=8). Values that have a different superscript letter (a,b,c,d) differ significantly with each other (P<0.05, Duncan's multiple range test).

Groups	Food intake (g/day)	Water intake (ml/day)
Normal control	54.37±3.29[a]	213.45±12.90[a]
Normal+rutin (25 mg/kg)	51.01±3.88[a]	218.04±16.60[a]
Normal+rutin (50 mg/kg)	56.01±3.27[a]	210.04±12.25[a]
Normal+rutin (100 mg/kg)	54.01±4.11[a]	226.04±17.21[a]
Diabetic control	68.01±5.18[b]	360.06±27.42[b]
Diabetic+rutin (25 mg/kg)	63.16±4.04[b,c]	343.42±21.99[b,c]
Diabetic+rutin (50 mg/kg)	64.36±4.35[b,c]	318.83±21.56[c,d]
Diabetic+rutin (100 mg/kg)	62.01±4.72[c]	310.05±23.61[d]

Mixed well and kept in dark for 5 min. and added 2.0 ml of n-butanol. The colour developed was read at 520 nm. Standard α-tocopherol in the range of 10–100 µg was taken and treated similarly along with blank containing only the reagent.

Estimation of ceruloplasmin. Plasma ceruloplasmin levels was estimated by the method of Ravin (1961). To 8.0 ml of acetate buffer (0.4 M, pH 5.5), added 0.05 ml of plasma, 1.0 ml of 0.5% p-phenylene diamine hydrochloride, mixed and kept at 37° for 1 hr. One ml of 0.5% sodium azide was added and kept at 4° for 30 min. A control tube was carried along with the test where sodium azide was added before the addition of p-phenylene diamine hydrochloride. The colour developed was read at 540 nm with control as blank.

Statistical analysis. All the grouped data were analysed by one way analysis of variance followed by Duncan's multiple range test using SPSS software package, version 9.05. The values are mean±S.D. for 8 rats in each group. P value<0.05 were considered as significant and included in the study.

Results

The initial and final body weight of normal and diabetic rats is given in table 1. A significant (P<0.05) decrease in the body weight of diabetic control rats (46.70%) was observed when compared with normal control rats. Diabetic + rutin (25, 50 and 100 mg/kg) treated rats showed a significant (P<0.05) increase in body weight (3.93%, 6.81% and 12.08% respectively) when compared with diabetic control rats. Rutin (25, 50 and 100 mg/kg) did not influence any significant (P<0.05) change in body weight in normal rats when compared to normal control rats.

The food and water intake in the experimental groups are presented in table 2. Diabetic control rats showed significantly (P<0.05) higher intake of food (25.09%) and water (40.71%) when compared with normal control group. The food intake was significantly (P<0.05) decreased in diabetic rats when treated with rutin (25, 50 and 100 mg/kg) (7.13%, 5.37% and 8.82% respectively), as well the water intake (4.62%, 11.45% and 13.89% respectively) when compared with diabetic control rats, but no significant (P<0.05) change was observed in rutin- (25, 50 and 100 mg/kg) treated normal rats.

Fasting plasma glucose levels of diabetic control rats reached as high as 21.17±1.61 mmol/l when compared with normal control rats (3.83±0.29 mmol/l) at the end of the experimental period (45 days). Rutin, orally administered at three different doses (25, 50 and 100 mg/kg) to diabetic rats significantly (P<0.05) decreased the plasma glucose levels (44.36%, 50.92% and 62.73% respectively) when compared to diabetic control rats at the end of the study. But, oral administration of rutin (25, 50 and 100 mg/kg) to normal rats did not show any significant (P<0.05) effect on fasting plasma glucose levels in this study (table 3). Rutin at a dose of 100 mg/kg exhibited a maximum plasma glucose lowering effect (62.73%) in diabetic rats than the other two

Table 3.

Effect of rutin on plasma glucose levels (mmol/l) in normal and diabetic rats on day 0, day 15, day 30 and on day 45. Each value is mean±S.D. for 8 rats in each group (n=8). Values that have a different superscript letter (a,b,c,d,e) differ significantly with each other (P<0.05, Duncan's multiple range test).

Groups	Day 0	Day 15	Day 30	Day 45
Normal control	3.94±0.20[a]	3.94±0.25[a]	4.11±0.17[a]	3.83±0.29[a]
Normal+rutin (25 mg/kg)	4.00±0.31[a]	4.11±0.20[a]	3.78±0.23[a]	3.97±0.22[a]
Normal+rutin (50 mg/kg)	3.78±0.19[a]	3.83±0.29[a]	3.94±0.21[a]	3.78±0.25[a]
Normal+rutin (100 mg/kg)	3.83±0.24[a]	3.94±0.30[a]	3.72±0.28[a]	3.89±0.30[a]
Diabetic control	15.78±1.20[b]	18.22±1.40[b]	20.33±1.50[b]	21.17±1.62[b]
Diabetic+rutin (25 mg/kg)	14.61±1.12[c]	15.22±1.17[c]	13.50±1.03[c]	11.78±0.94[c]
Diabetic+rutin (50 mg/kg)	15.56±1.15[b]	14.00±1.07[d]	12.22±0.94[d]	10.39±0.70[d]
Diabetic+rutin (100 mg/kg)	14.94±1.12[b,c]	12.94±0.92[d]	10.78±0.83[e]	7.89±0.60[e]

Table 4.

Effect of rutin on plasma insulin, C-peptide, total haemoglobin, glycosylated haemoglobin and total protein levels in normal and diabetic rats. Each value is mean±S.D. for 8 rats in each group (n=8). Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05, Duncan's multiple range test).

Groups	Insulin (μU/ml)	C-peptide (ng/ml)	Total haemoglobin (g %)	Glycosylated haemoglobin (mg/dl)	Total proteins (g/dl)
Normal control	13.67±1.04[a]	22.90±1.70[a]	10.31±0.62[a]	0.41±0.03[a]	10.60±0.81[a]
Normal+rutin (100 mg/kg)	13.74±1.05[a]	22.41±0.92[a]	10.33±0.79[a]	0.39±0.03[a]	10.72±0.82[a]
Diabetic control	6.89±0.22[b]	12.10±1.33[b]	6.51±0.57[b]	0.80±0.06[b]	6.80±0.52[b]
Diabetic+rutin (100 mg/kg)	10.92±0.48[c]	18.42±1.71[c]	9.83±0.69[c]	0.49±0.04[c]	9.34±0.64[c]

doses (25 and 50 mg/kg) at the end of the experiment. Hence, further studies were carried out with 100 mg/kg of rutin alone in normal and streptozotocin-diabetic rats.

Significantly (P<0.05) decreased levels of plasma insulin (49.60%), C-peptide (45.73%), total proteins (35.85%) and total haemoglobin (37.28%) and increased levels of glycosylated hemoglobin (95.12%) were observed in diabetic control rats when compared with normal control rats (table 4). Oral administration of rutin to diabetic rats significantly (P<0.05) increased plasma insulin (58.49%), C-peptide (52.23%), total proteins (37.35%), total haemoglobin (51%) and decreased glycosylated haemoglobin (38.75%) when compared with diabetic control rats.

In diabetic control rats, plasma thiobarbituric acid reactive substances and hydroperoxides increased significantly (P<0.05) (74.41% and 102.5% respectively) on comparison with normal control rats. Diabetic rats when treated with rutin, exhibited a significant (P<0.05) effect on plasma thiobarbituric acid reactive substances and hydroperoxides and decreased their levels (28.26% and 39.51% respectively) when compared with diabetic control rats (table 5).

The levels of non-enzymic antioxidants in normal and diabetic rats is given in table 6. There was a significant (P<0.05) decrease in the levels of GSH (44.78%), vitamin C (60%), vitamin E (33.33%) and ceruloplasmin (33.33%) in diabetic control rats compared to normal control rats. Oral administration of rutin to diabetic rats lead to a significant (P<0.05) increase in the plasma levels of GSH (56.76%), vitamin C (125%), vitamin E (50%) and ceruloplasmin (36.67%) when compared with diabetic control rats.

Table 5.

Effect of rutin on plasma thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in normal and diabetic rats. Each value is mean±S.D. for 8 rats in each group (n=8). Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05, Duncan's multiple range test).

Groups	TBARS (nmol/ml)	HP (nmol/ml)
Normal control	2.11±0.16[a]	0.80±0.01[a]
Normal+rutin (100 mg/kg)	2.08±0.15[a]	0.77±0.01[a]
Diabetic control	3.68±0.28[b]	1.62±0.01[b]
Diabetic+rutin (100 mg/kg)	2.64±0.22[c]	0.98±0.01[c]

Treatment of normal rats with rutin (100 mg/kg) did not significantly (P<0.05) alter any of the parameters studied.

Discussion

Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes (Baynes 1991). Increased free radical production or reduced antioxidant defense responses, both of which occur in the diabetic state may give rise to increased oxidative stress (Halliwell & Gutteridge 1990). Consequences of oxidative stress are adaptation or cell injury, i.e. damage to DNA, proteins and lipids, disruption in cellular homeostasis and accumulation of damaged molecules (Jakus 2000). Reduced oxidative stress in the diabetic condition has been observed in experimental animals after the administration of certain polyphenols (Sanders *et al.* 2001).

In streptozotocin-induced diabetic rats, increased food consumption and decreased body weight were observed. This indicates polyphagic condition and loss of weight due to excessive break-down of tissue proteins (Chatterjea & Shinde 2002). Hakim *et al.* (1997) have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats (Rajkumar *et al.* 1991). Rutin administration to diabetic rats decreased food consumption and improved body weight and this could be due to a better control of the hyperglycaemic state in the diabetic rats. Decreased levels of blood glucose could improve body weight in streptozotocin-diabetic rats (Kamalakkanan *et al.* 2003; Babu & Stanely Mainzen Prince 2004).

Streptozotocin administration to rats increased blood glucose and decreased insulin and C-peptide levels. Rutin-treated streptozotocin-diabetic rats exhibited a decrease in plasma glucose and an increase in insulin and C-peptide levels. Rutin by its ability to scavenge free radicals and to inhibit lipid peroxidation, prevents streptozotocin-induced oxidative stress and protects β-cells resulting in increased insulin secretion and decreased blood glucose levels. In this context, research by Vessal *et al.* (2003) have shown that quercetin, the aglycone of rutin decreased blood glucose concentration and increased insulin release in streptozotoc-

Table 6.

Effect of rutin on plasma glutathione, vitamin C, vitamin E and ceruloplasmin in normal and diabetic rats. Each value is mean±S.D. for 8 rats in each group (n=8). Values that have a different superscript letter (a,b,c) differ significantly with each other ($P<0.05$, Duncan's multiple range test).

Groups	Glutathione (mmol/l)	Vitamin C (mmol/l)	Vitamin E (mmol/l)	Ceruloplasmin (nmol/l)
Normal control	0.67 ± 0.05^{a}	0.10 ± 0.01^{a}	0.03 ± 0.002^{a}	1.35 ± 0.10^{a}
Normal+rutin (100 mg/kg)	0.67 ± 0.04^{a}	0.11 ± 0.01^{a}	0.03 ± 0.002^{a}	1.36 ± 0.10^{a}
Diabetic control	0.37 ± 0.02^{b}	0.04 ± 0.01^{b}	0.02 ± 0.001^{b}	0.90 ± 0.07^{b}
Diabetic+rutin (100 mg/kg)	0.58 ± 0.04^{c}	0.09 ± 0.01^{c}	0.03 ± 0.002^{c}	1.23 ± 0.08^{c}

in-induced diabetic rats. Coskun *et al.* (2005) have also reported that, in streptozotocin-induced diabetic rats, quercetin protected pancreatic β-cells by decreasing oxidative stress and preserving pancreatic β-cell integrity. Increased insulin levels could also be due to the stimulatory effect of rutin, thereby potentiating the existing β-cells of the islets of Langerhans in diabetic rats. Hii & Howell (1984 & 1985) showed increased number of pancreatic islets in quercetin-treated animals.

C-peptide is formed in the biosynthesis of insulin, and the two peptides (insulin and C-peptide) are subsequently released in equimolar amounts to the circulation (Wahren 2001). An increase in C-peptide levels in diabetic rats treated with rutin correlates well with the increased insulin secretion (endogenous secretion) thereby possibly regenerating β-cells of the islets of Langerhans. Chakravarthy *et al.* (1980 & 1983) have demonstrated selective regeneration of β-cells of alloxan-damaged pancreas by administering a flavonoid fraction of *Pterocarpus marsupium* and (-) epicatechin.

Under conditions of severe oxidative stress, free radical generation leads to protein modification. Proteins may be damaged directly by specific interactions of oxidants or free radicals with particularly susceptible amino acids. They are also modified indirectly, with reactive carbonyl compounds formed by the auto-oxidation of carbohydrates and lipids, with eventual formation of advanced glycation/lipoxidation end products (Gumieniczek 2005). In diabetes mellitus, a variety of proteins are subjected to non-enzymatic glycation and is thought to contribute to the long-term complication of the disease (Vlassara *et al.* 1981). The levels of plasma total proteins were found to be decreased in this study. This could be due to increased lipid peroxidation in the diabetic rats. The decrease in the total proteins concentration in serum of diabetic rats may also be ascribed to (i) decreased amino acids uptake, (ii) greatly decreased concentration of variety of essential amino acids, (iii) increased conversion rate of glycogenic amino acids to CO_2 and H_2O and (iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA (Ahmed 2005). Decreased protein content of blood serum in diabetic patients were reported by Mahboob *et al.* (2005) indicating elevated lipid peroxidation process and decreased antioxidant defensive system.

Measurement of glycated haemoglobin has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein 1995). The glycated haemoglobin levels increased in diabetic rats with a subsequent decrease in the levels of haemoglobin. Agents with antioxidant or free radical scavenging power may inhibit oxidative reactions associated with glycation (Elgawish *et al.* 1996). Rutin with its free radical scavenging capability effectively reduced the formation of glycated haemoglobin and increased the haemoglobin levels in diabetic rats. A decrease in blood glucose levels might also contribute to decreased levels of glycated haemoglobin in rutin-treated diabetic rats. Rutin possesses antioxidant activity that includes an affinity to scavenge free radicals (Liao & Yin 2000). The protective effect of rutin against haemoglobin oxidation was reported by Grinberg *et al.* (1994). Nagasawa *et al.* (2003a) have shown that rutin and G-rutin (a water soluble rutin analogue) suppressed the formation of both initial and advanced stages of Maillard reaction in tissue (muscle and kidney) protein sources. They (Nagasawa *et al.* 2003b) have also shown that G-rutin suppressed the accumulation of glycation products in serum and tissue (kidney) protein sources, attributing these to the antioxidant capacity of rutin.

Lipid peroxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants (Korkina & Afanas'ev 1997). Lipid peroxidation end products measured as thiobarbituric acid reactive substances and hydroperoxides were seen increased in plasma of streptozotocin-diabetic rats in this study. Drugs with antioxidant properties may supply endogenous defense systems and reduce both initiation and propagation of reactive oxygen species (Bergendi *et al.* 1999). Rutin effectively reduced the increased levels of thiobarbituric acid reactive substances and hydroperoxides in diabetic rats. In this context, rutin has been shown to suppress lipid peroxidation *in vitro* (Kozlov *et al.* 1994).

The massive production of reactive species may also lead to the depletion of protective physiological moieties such as glutathione, vitamin C, vitamin E and ceruloplasmin in diabetic rats. Administration of rutin to diabetic rats increased the levels of non-enzymic antioxidants. Decreased oxidative stress in diabetic rats treated with rutin could result in the restoration of glutathione levels in the plasma. Glutathione through its significant reducing power contrib-

utes to the recycling of other antioxidants such as vitamin C and vitamin E, that have become oxidized (Kidd 1997). Rutin was reported to potentiate the antilipoperoxidative capacity of vitamin C and vitamin E (Negre-Salvayre *et al.* 1991). The capability of rutin to form metal ion chelates might lead to an increase in the levels of ceruloplasmin. An increase of the antioxidant capacity of plasma indicates absorption of antioxidants and an improved *in vivo* antioxidant status (Cao 1998), or an adaptation mechanism to an increased oxidative stress.

Rutin possesses suitable pharmacokinetic properties. On oral administration, rutin can be hydrolyzed by the intestinal microflora with α-rhamnosidase and β-glucosidase to isoquercitrin (quercetin 3-glucoside) and quercetin (Bokkenheuser *et al.* 1987). Then, quercetin is absorbed and the absorbed quercetin is excreted into the bile and urine as glucuronide and sulfate conjugates within 48 hr (Ueno *et al.* 1983). It has been reported that quercetin is further degraded as phenolic acids such as 3-hydroxyphenylacetic acid and 3,4-dihydroxy phenyl acetic acid through B-ring fission by intestinal bacteria (Hollman & Katan 1998). Pharmacokinetic studies of rutin were carried out in healthy volunteers by Boyle *et al.* (2000) and Erlund *et al.* (2000). Rutin supplementation for six weeks resulted in increased concentrations of quercetin, kaempferol and isorhamnetin (Boyle *et al.* 2000). In the study by Erlund *et al.* (2000), after 32 hr of supplementation of quercetin and rutin, these compounds were found in plasma as glucuronides and/or sulfates of quercetin and as unconjugated quercetin aglycone. No rutin was detected.

Numerous studies indicate that dietary supplementation with antioxidant nutrients may be a safe and simple complement to traditional therapies for preventing and treating diabetic complications (Ruhe & McDonald 2001). Rousselot (2004) stated that improved antioxidant status may be one mechanism by which dietary antioxidant treatment contributes to the prevention and reduction of diabetic complications including blindness, kidney failure, limb amputation and even death. Quercetin has been shown to attenuate diabetic nephropathy in streptozotocin-diabetic rats (Anjaneyulu & Chopra 2004). Consumption of flavonoid-containing foods and beverages have been proposed as a useful practice to limit oxidative damage in the body. The antihyperglycaemic and antioxidant activity of rutin is evident from our present study. Further experimental and clinical studies are warranted before rutin could be used as a supplement for the treatment of diabetes mellitus and its complications.

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