Chapter I

Antihyperglycemic effect of naringenin in Wistar rats with streptozotocin-nicotinamide-induced diabetes mellitus

1. Introduction

Diabetes mellitus, a pervasive and multifactorial chronic metabolic disorder, is characterized by defects in endogenous insulin secretion or action, or both, which results in chronic hyperglycemia, a clinical hallmark of diabetes, often accompanied by glycosuria, polydipsia polyuria and weight loss (Maritim et al., 2003). Chronic hyperglycemia eventually leads to progressive beta-cell dysfunction, impaired insulin gene transcription and permanent β-cell loss due to apoptosis (Brunner et al., 2009; Poitout et al., 2010).

During the postprandial state, insulin secretion from the pancreatic β-cells controls systemic nutrient homeostasis by promoting anabolic processes including glucose oxidation for adenosine triphosphate (ATP) generation, fat, protein and glycogen synthesis and storage, inhibition of fatty acid oxidation, glycogenolysis, and gluconeogenesis, as well apoptosis and autophagy in insulin-responsive tissues (Saltiel and Kahn, 2001; Fritsche et al., 2008). During the fasting state, insulin secretion decreases, and tissues coordinate with counter-regulatory hormones, such as glucagon, in favor of using fatty acids largely for ATP generation and maintenance of glucose homeostasis (Cahill, 2006). The substrate preferences for metabolic adaptation, during the transit from fasting to the postprandial state, are tightly controlled by insulin under physiological conditions (Randle, 1963). This adaptive transition reflects the action of insulin in insulin-responsive organs, while it is largely blunted in organs with insulin resistance, preceding the development of Type 2 diabetes (Johnson and Olefsky, 2013).
Consequently, in the diabetic state, alterations in insulin secretion and its action lead to impaired glucose homeostasis and the occurrence of chronic hyperglycemia, which leads to a reduced number of glucose transporters, down regulation in the number of insulin receptors as well as defects of tissue insulin signal transduction, all of which results in insulin resistance (Kido et al., 2000). Insulin initiates a wide variety of growth and metabolic effects by binding to the insulin receptor (IR) and activates the insulin receptor tyrosine kinase, which phosphorylates and recruits different substrate adaptors such as the insulin receptor substrate (IRS) family of proteins. Phosphorylated IRS proteins serve as multisite docking proteins for various effector molecules possessing src homology 2 (SH2) domains, including phosphatidylinositol-3-kinase (PI3K) regulatory subunits, the tyrosine kinases, as well as several smaller adapter molecules such as the growth factor receptor binding proteins (White, 2003; Sun and Liu, 2009). IRS proteins function as essential signaling intermediates downstream of activated cell surface insulin receptors, and play a central role in maintaining basic insulin-mediated cellular functions including glycogen synthesis, protein synthesis, cell survival and glucose uptake, and promote fatty acid synthesis and inhibit gluconeogenesis (White, 2003; Berg et al., 2007; Fritsche et al., 2008). Insulin resistance occurs at multiple levels in cells, from the cell surface to the nucleus, including insulin receptor desensitization, suppression of IRS protein and functionality, inhibition of PI3K cascades, and failure to restrain fork head box protein O1 (FOXO1)-activated gene transcriptional profiling, all of which can result from inhibition of IRS-1 and IRS-2 (Valverde et al., 2003; Guo, 2013).

Insulin stimulates amino acid uptake into cells, inhibits protein degradation and promotes protein synthesis (Saltiel and Kahn, 2001). Its deprivation in the diabetic state causes a profound increase in protein catabolism, mainly due to a net increase in protein breakdown rather than a decline in protein synthesis (Moller and Nair, 2008).
The alterations in protein metabolism may be responsible for many of the chronic complications of diabetes, since they may involve both structural and functional proteins (Sugden and Fuller, 1991). Furthermore, chronic hyperglycemia itself has negative impacts on various organs, especially the eyes, kidneys, nerves, (Park et al., 2008) heart, and blood vessels, that could lead to micro- and macro-vascular complications, including cardiovascular disease (CVD), retinopathy, nephropathy, and neuropathy (Maritim et al., 2003; Folli et al., 2011).

Naringenin is a flavonone compound found in citrus fruits, such as grapes and oranges, and also in tomato skin. Naringenin has been pharmacologically evaluated for antioxidant (Baumann et al., 1980), anti-inflammatory (Shi et al., 2009), anticancerous (Park et al., 2008), anti-atherosclerotic (Wilcox et al., 1999), hepatoprotective (Yen et al., 2009), nephroprotective (Badary et al., 2005) and immuno-modulatory (Du et al., 2009) activities. Recently, Ortiz-Andrade et al. (2008) reported that naringenin is nontoxic, with a high (5,000 mg/kg) LD50, and that it could prevent the absorption of glucose from the intestine in rats with non-insulin dependent diabetes mellitus. However, a review of the literature reveals a paucity of data on the antidiabetogenic effect of naringenin on the pancreas and liver, the two major organs that play a critical role in glucose homeostasis via secretion and clearance, respectively, of insulin. In the present chapter, an attempt was made to evaluate the putative anti-hyperglycemic effect of naringenin in rats with experimental streptozotocin (STZ)-nicotinamide-induced diabetes mellitus through various biochemical as well as molecular approaches.

2. Materials and methods

Animals, grouping of animals and treatment regimen used in this phase of the study were as described in the General Materials and Methods section (Pages 37 – 39). The body weight of the experimental animals was recorded throughout the study.
2.1. Oral glucose tolerance test

At the end of the experimental period, fasting blood samples (collected in EDTA) were taken from all groups of rats. In addition, blood samples (in EDTA) were collected at 30, 60, 90 and 120 min intervals after administration of a glucose solution (2 gms/kg b. w.).

2.2. Preparation of tissue homogenate

Hepatic tissues from each experimental animal were homogenized (100 mg/ml buffer) with 50 mM phosphate buffer (pH 7.0) and centrifuged at 12,000 xg for 15 min. at 4°C. The supernatant thus obtained was used for immunoblot and biochemical assays. The protein concentration of each fraction was determined by the method of Bradford (1976), using crystalline bovine serum albumin as standard.

2.3. Biochemical assays

2.3.1. Determination of blood glucose, glycosylated hemoglobin and serum insulin

Blood glucose was assayed by the glucose oxidase-peroxidase (GOD-POD) method, using a commercial kit (ERBA, Mumbai, India). Glycosylated hemoglobin was assayed in the blood by the chromatographic-spectrophotometric ion-exchange method, using a commercial diagnostic kit (Biosystems S.A., Barcelona, Spain). Serum insulin levels were assayed by solid phase enzyme-linked immunosorbent assay, using a commercial kit (UBI Magiwel, Mountain View, Canada).

2.3.2. Determination of blood urea and serum advanced glycation end-products (AGES), creatinine and uric acid

Serum advanced glycation end-products were assayed by enzyme-linked immunosorbent assay, using a commercial kit (Cell Biolabs, San Diego, CA) and expressed as mU. Blood urea (Natelson et al., 1951), serum creatinine (Brod and Sirota,
1948), and uric acid (Caraway, 1963) were also determined spectrophotometrically and expressed as mg/dl.

2.3.3. **Determination of activities of hepatic marker enzymes in serum**

Activities of AST and ALT were determined (King, 1965a) and expressed in terms of μmoles of pyruvate liberated/min/mg of protein at 37°C. ALP activity was assayed using disodium phenyl phosphate as substrate (King, 1965b) and expressed as μmoles of phenol liberated/min/mg of protein. LDH was assayed by the method of (King, 1965c), in which LDH convert lactate to pyruvate with the help of the coenzyme nicotinamide adenine dinucleotide (NAD), the pyruvate formed was made to react with dinitrophenyl hydrazine in HCl, the resulting hydrazone turning into an orange-coloured complex in alkaline medium, which was measured at 420 nm.

2.3.4. **Immunoblot**

Immunoblot analyses were performed to determine the relative concentrations of IRS-1 and IRS-2 in samples of the various groups. Proteins subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were electrophoretically transferred to a poly vinylidene fluoride (PVDF) membrane using a semi-dry blotting apparatus (Bio-Rad, Hercules, CA, USA). Blotting was done at 24 V for 1 h. Blotted membranes were stained by Ponceau S solution to check for the efficiency of transfer; subsequently, blocking was done with 5% non-fat dry milk in Tris buffered saline (pH 7.5) with 0.1 % (v/v) Tween 20 for 2 h. Antibodies against IRS-1 (1:200 dilutions) and IRS-2 (1:200 dilutions) [both purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA] were used. Immuno-reactivity was visualized with alkaline phosphatase conjugated to anti-mouse IgG secondary antibodies and 5-bromo 4-chloro 3-indolyl phosphate/nitroblue tetrazolium chloride [BCIP/NBT (Genei, Bangalore, India)]. To analyze the minimal changes observed in the intensity of the bands, densitometry was performed on the scanned images of the
membrane. The program Quantity One software (Bio-Rad, Hercules, CA, USA) was used for the analysis of intensity of bands in each lane of the membrane.

2.3.5. **Statistical analysis**

The values are expressed as mean ± standard deviation (SD) for six animals in each group. The statistical significance of differences between all the groups was assessed by one-way analysis of variance (ANOVA) using SPSS software package for Windows (Version 16.0; IBM Corporation, Armonk, NY). Where ANOVA yielded significant results, post-hoc testing was performed for inter-group comparisons (between two groups) using the least significant difference (LSD) test. Values were considered statistically significant when P<0.05.

3. **Results**

3.1. **Change in body weight**

During the experimental period, there was a significant (P<0.05) decrease in body weight of diabetic, untreated rats (Group III) when compared with normal (Group I) and normal rats treated with naringenin (Group II) (Figure 1.1). However, this significant (P<0.05) weight loss was prevented in diabetic rats administered naringenin (Group IV) and those administered glyclazide (Group V).

3.2. **Assay of oral glucose tolerance test**

Blood glucose concentrations in normal (Group I) rats and normal rats treated with naringenin (Group II) increased to a maximum value at 60 min after the oral glucose load and declined to near basal levels at 120 min; however, in diabetic, untreated (Group III) rats, the peak increase in blood glucose level was noted after 60 min and remained high over the next 60 min. In diabetic rats administered naringenin (Group IV) and those administered glyclazide (Group V), significantly (P<0.05) lower blood glucose levels were noted at 60 min when compared with diabetic, untreated
Figure 1.1. Body weight changes in Experimental Groups of Wistar Rats

Values represent the mean ± SD of observations made on six rats in each group.

Group I - normal untreated; Group II - normal, naringenin-treated; Group III - diabetic untreated; Group IV - diabetic naringenin-treated; Group V - diabetic glyclazide-treated

a Statistically significant difference (P<0.05) when compared with Group I and Group II values.

b Statistically significant difference (P<0.05) when compared with Group III values.
(Group III) rats. No significant differences were observed in these test parameters between Group IV and V rats. There were also no significant differences between the mean blood glucose levels in Group I and II rats (Figure 1.2).

3.3. Assay of blood glucose levels, glycosylated hemoglobin percentages and serum insulin levels in Wistar rats

Table 1.1 lists the mean blood glucose levels, glycosylated hemoglobin percentages and serum insulin levels in each of the five groups of rats. Normal rats treated with naringenin (Group II) did not exhibit significant differences in the test parameters when compared to untreated normal (Group I) rats. Diabetic, untreated (Group III) rats showed significantly (P<0.05) higher mean levels of blood glucose and glycosylated hemoglobin and significantly (P<0.05) lower mean levels of serum insulin, when compared to untreated normal (Group I) and naringenin- treated normal (Group II) rats. Conversely, diabetic rats administered naringenin (Group IV) and those administered glyclazide (Group V) exhibited significantly (P<0.05) lower mean levels of blood glucose and glycosylated hemoglobin and significantly (P<0.05) higher mean levels of serum insulin, when compared to diabetic, untreated (Group III) rats. No significant differences were noted between the values obtained in naringenin-treated (Group IV) rats and those in glyclazide-treated (Group V) rats. However, although there were significant improvements in these parameters in Group IV and Group V rats (compared to the levels in diabetic untreated rats), the values in Group IV and Group V rats still differed significantly from those observed in the normal untreated (Group I) and normal naringenin-treated (Group II) rats.

3.4. Assay of serum AGEs, creatinine, uric acid and blood urea levels in Wistar rats

Table 1.2 lists the mean serum AGEs, creatinine, uric acid and blood urea levels in each of the five groups of rats. Normal rats treated with naringenin (Group II) did not
Table 1.1. Mean blood glucose levels, glycosylated hemoglobin percentages and serum insulin levels in Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Blood glucose (mg dl⁻¹)</th>
<th>Glycosylated Hemoglobin (% of hb)</th>
<th>Serum insulin (μU ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>91.5±3.37</td>
<td>6.74±0.44</td>
<td>13.59±0.62</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>89.14±2.3</td>
<td>6.26±0.49</td>
<td>13.24±0.36</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>325.28±8.63ᵃ</td>
<td>11.38±0.61ᵃ</td>
<td>6.97±0.85ᵃ</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>118.26±5.74ᵃᵇ</td>
<td>7.8±0.47ᵃᵇ</td>
<td>12.01±0.53ᵃᵇ</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>116.18±9.71ᵃᵇ</td>
<td>7.51±0.47ᵃᵇ</td>
<td>12.16±0.47ᵃᵇ</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD of observations made on six rats in each group.

Statistical analysis: one way analysis of variance [ANOVA] with post-hoc testing [least significant difference]

ᵃ Statistically significant difference (P<0.05) when compared with Group I and Group II values.

ᵇ Statistically significant difference (P<0.05) when compared with Group III values.
Table 1.2.  Mean level of serum advanced glycation end-products, blood urea, serum creatinine and serum uric acid in Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Serum Advanced glycation end products (mU)</th>
<th>Blood urea (mg dl⁻¹)</th>
<th>Serum creatinine (mg dl⁻¹)</th>
<th>Serum uric acid (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>5.24±0.23</td>
<td>25.5±1.27</td>
<td>1.24±0.64</td>
<td>3.29±0.82</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>5.32±0.23</td>
<td>25.14±0.92</td>
<td>1.28±0.69</td>
<td>3.24±0.96</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>8.02±0.3ᵃ</td>
<td>39.14±1.2ᵃ</td>
<td>2.42±0.61ᵃ</td>
<td>5.97±0.85ᵃ</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>6.1±0.16ᵃᵇ</td>
<td>28.72±1.2ᵃᵇ</td>
<td>1.38±0.67ᵃᵇ</td>
<td>4.01±0.83ᵃᵇ</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>6.0±0.16ᵃᵇ</td>
<td>27.28±1.71ᵃᵇ</td>
<td>1.31±0.57ᵃᵇ</td>
<td>3.96±0.74ᵃᵇ</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD for observations made on six rats in each group.

Statistical analysis: one way analysis of variance [ANOVA] with post-hoc testing [least significant difference]

ᵃ Statistically significant difference (P<0.05) when compared with Group I and Group II values.

ᵇ Statistically significant difference (P<0.05) when compared with Group III values.
exhibit significant differences in the test parameters when compared to untreated normal (Group I) rats. The mean levels of these test parameters in diabetic, untreated (Group III) rats were significantly (P<0.05) higher, when compared to those in untreated normal (Group I) and naringenin- treated normal (Group II) rats (Table 1.2). Conversely, diabetic rats administered naringenin (Group IV) and those administered glyclazide (Group V) exhibited significantly (P<0.05) lower mean levels, when compared to diabetic, untreated (Group III) rats. No significant differences were noted between the values obtained in naringenin-treated (Group IV) rats and those in glyclazide-treated (Group V) rats. However, although there were significant improvements in these parameters in Group IV and Group V rats (compared to the levels in diabetic untreated rats), the values in Group IV and Group V rats still reminded significantly higher than those observed in the normal untreated (Group I) and normal naringenin-treated (Group II) rats.

3.5. Activities of hepatic marker enzymes in serum of Wistar rats

The mean activities of serum ALT, AST, ALP and LDH were found to be significantly (P<0.05) higher in Group III rats than those in Group I and Group II rats. Although rats in Groups IV and V exhibited significantly (P<0.05) lower mean levels of these enzymes than did Group III rats, the mean activities were still significantly higher than those in Group I and Group II rats. The mean activities of these enzymes in serum were significantly higher in Group IV rats than those in Group V rats. No significant differences were observed in these test parameters between Group I and Group II rats (Figure 1.3).

3.6. Immunoblot analysis of IRS-1 and -2 proteins

The expression of the IRS-1 and -2 proteins in the hepatic tissue samples from the five groups of rats was analyzed by immuno-blotting (Figure 1.4). The intensities of the bands corresponding to these proteins were significantly (P<0.05) lower in Group
Values represent the mean ± SD of observations made on six rats in each group.

The enzyme activities are expressed thus: i) ALT and AST as: μmoles of pyruvate liberated/h/mg of protein; ii) ALP- μmoles of phenol liberated/min/mg of protein; iii) LDH- μmoles of pyruvate formed/ h/ mg protein.

Abbreviations: AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=alkaline phosphatase; LDH=Lactate dehydrogenase

Group I - normal untreated; Group II - normal, naringenin-treated; Group III - diabetic untreated; Group IV - diabetic naringenin-treated; Group V - diabetic glyclazide-treated

Statistical analysis: one way analysis of variance [ANOVA] with post hoc testing [least significant difference]

a Statistically significant difference (P<0.05) when compared with Group I and Group II values.

b Statistically significant difference (P<0.05) when compared with Group III values.

c Statistically significant difference (P<0.05) when compared with Group V values
Figure 1.4.  

a) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

b) Immunoblot demonstrating expression of IRS-1 and 2 in the hepatic tissue. The β-actin antibody was used as loading control

c) Bar graphs of mean normalized densitometry for these data.

M, Protein Marker; Group I - Normal untreated; Group II - Normal, naringenin-treated; Group III - Diabetic untreated; Group IV - Diabetic naringenin-treated; Group V - Diabetic glyclazide-treated

Abbreviation: IRS = Insulin Receptor Substrate

a Statistically significant difference (P<0.05) when compared with Group I and Group II values

b Statistically significant difference (P<0.05) when compared with Group III values
III rats, when compared to Group I and Group II rats. Conversely, rats in Groups IV and V exhibited significantly (P<0.05) higher mean levels of protein band intensity than those in Group III rats. No significant differences in the mean levels of protein band intensity were noted between Group IV and V rats. There were also no significant differences between the mean level of intensities in Group I and II rats (Figure 1.4).

4. Discussion

Streptozotocin (STZ), N-(methyl nitro carbamoyl)-D-glucosamine is an antibiotic derived from Streptomyces achromogenes. This compound is universally used to induce diabetes mellitus in experimental animals due to its cytotoxic effects on pancreatic β-cells (Kajimoto and Kaneto, 2004). Nicotinamide can partially reverse the inhibition of insulin secretion to prevent aggravation of experimental diabetes following the administration of STZ (LeDoux et al., 1988). Animals with STZ-nicotinamide-induced diabetes mellitus exhibit a number of attributes that are similar to those found in humans suffering from non-insulin dependent diabetes mellitus, such as persistent hyperglycemia, glucose intolerance and significantly altered glucose-stimulated secretion of insulin (Masiello et al., 1998). Hence, in the present study, male rats with experimental STZ-nicotinamide-induced diabetes mellitus were used as the experimental model to evaluate the antihyperglycemic effect of naringenin.

The destruction of β-cells during diabetes ultimately causes physico-metabolic abnormalities such as a decrease in body weight, and increase in food and water intake (Rodriguez et al., 1997). In the present study, the observed decrease in body weight of diabetic, untreated rats when compared with normal (Group I) and normal rats treated with naringenin (Group II) rats may have been due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis, thereby increasing protein deterioration for energy metabolism that could lead to increased muscle destruction or degradation of structural proteins, as hypothesized earlier (Salahuddin
and Jalalpure, 2010; Oyedemi et al., 2011). However, a significant improvement in body weight was noted in diabetic rats administered naringenin (Figure 1.1), suggesting that naringenin may increase glucose uptake and its utilization by the cells for energy metabolism thereby preventing protein wasting which, in turn, promotes gain in body weight. Recently, Palsamy and Subramanian (2008) reported a similar phenomenon in STZ-nicotinamide-induced rats with diabetes mellitus treated with resveratrol, a natural phytoalexin.

In STZ-nicotinamide-induced diabetic untreated (Group III) rats, significantly (P<0.05) higher mean blood glucose levels and significantly (P<0.05) lower mean serum insulin levels were noted than the values in normal (Group I) rats (Table 1.1). However, administration of naringenin to STZ-nicotinamide-induced rats with diabetes mellitus appeared to maintain blood glucose and serum insulin levels at near normal levels (Table 1.1). Naringenin possibly stimulated insulin secretion from the remaining pancreatic β-cells, thereby exerting its antihyperglycemic action in experimental diabetes. Interestingly, it has recently been shown that naringenin improves insulin signaling and sensitivity in a dietary model of metabolic syndrome (Kannappan and Anuradha, 2010); moreover, naringenin has been found to prevent hepatic steatosis and to improve insulin sensitivity in animals fed a high fat diet (Mulvihill et al., 2009).

An oral glucose tolerance test is a sensitive measure of early abnormalities in carbohydrate metabolism during post glucose administration (Ceriello, 2005). Impaired glucose tolerance reflects reduced uptake of glucose from blood into liver, skeletal muscle and adipose tissue following a meal and increased hepatic gluconeogenesis (Robertson, 2007). Impaired glucose tolerance serves as a marker for the state of insulin resistance and predicts both large and small-vessel vascular complications (Tominaga et al., 1999). In the present study, the impaired glucose tolerance observed in diabetic group of rats was significantly improved in diabetic rats treated with
naringenin (Figure 1.2), suggesting that naringenin may stimulate insulin effects and increase glucose transport and utilization, thereby maintaining the glucose homeostasis.

In persistent hyperglycemia, the excess blood glucose reacts non-enzymatically with hemoglobin to form glycosylated hemoglobin, which is a reliable marker of ambient glycemia over a period of 90 days (al-Shamaony et al., 1994). In the present investigation, significantly higher mean percentages of glycosylated hemoglobin were observed in diabetic untreated rats than in normal untreated rats (Table 1.1). However, lower mean percentages of blood glycosylated hemoglobin were observed in naringenin-treated diabetic rats, probably due to the reduction in mean blood glucose levels brought about by naringenin. Other investigators have also hypothesized that lowered levels of glycosylated hemoglobin are the consequence of reduced blood glucose levels (Kaleem et al., 2006; Ramesh et al., 2006; Palsamy and Subramanian, 2008). Punithavathi et al. (2011) recently reported that in rats with streptozotocin-induced diabetes mellitus, administration of gallic acid resulted in decreased levels of blood glucose which, in turn, resulted in lowered levels of glycosylated hemoglobin.

In diabetes mellitus, hyperglycemia increases protein glycation and the formation of AGEs, accompanied by increased free radical activity that contributes towards the cell death and renal dysfunction in diabetes (Brownlee et al., 1984). Studies have shown that hyperglycemia-mediated oxidative stress leads to thickening of glomerular basement membranes and expansion of mesangial cells (Forbes et al., 2008). These changes lead to hypertrophy and decrease in physiological functions of the kidney as reflected in increase in serum creatinine, uric acid and blood urea levels, which are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). Furthermore, the accumulation of urea, an end-product of protein catabolism in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins (Green and Miller, 1960). Interestingly, in the present study, the mean levels
of serum AGEs, creatinine, uric acid and blood urea were significantly (P<0.05) higher in diabetic, untreated (Group III) rats, when compared to normal (Group I) rats. However, diabetic rats administered naringenin (Group IV) exhibited significantly (P<0.05) lower mean levels of serum AGEs, creatinine, uric acid and blood urea, when compared to diabetic, untreated (Group III) rats (Table 1.2), suggesting that naringenin probably decreases hyperglycemia-mediated in glycation and catabolism of proteins, thereby averting increased oxidative stress and kidney dysfunction.

The liver, a major site of insulin clearance and production of inflammatory cytokines, plays an important role in maintaining normal glucose concentrations in fasting and post-prandial states (Leclercq et al., 2007). Elevated levels of serum enzymes, such as ALT, AST, ALP and LDH, are well-known markers of hepatic damage as these enzymes are believed to leak from the cytosol into the bloodstream as a consequence of damage to hepatic tissue (Zimmerman and Seef, 1970). In the present study, significantly (P<0.05) higher mean serum levels of ALT, AST, ALP and LDH were observed in STZ-nicotinamide-induced diabetic untreated (Group III) rats than in untreated normal (Group I) rats (Figure 1.3). However, in naringenin-treated diabetic (Group IV) rats, significantly (P<0.05) lower (compared to Group III) mean levels of these serum enzymes were noted (Figure 1), suggesting that naringenin may protect the hepatic tissue from hyperglycemia-mediated cellular damage and may increase insulin action.

Insulin and its signaling cascade normally control cell growth, metabolism and survival through activation of mitogen-activated protein kinases (MAPKs) and PI3K, of which activation of PI3K is associated with IRS-1 and -2 (Alessi et al., 1997; White, 2003; Fafalios et al., 2011). Recently, Guo et al. (2009) reported that suppression of IRS-1 and IRS-2 leads to inhibition of hepatic Akt→Foxo1 phosphorylation and resulted in the development of insulin resistance, hyperglycemia and hypolipidemia,
serving as important mechanism for development of Type 2 diabetes mellitus. A review of recent literature suggests that targeting the IRS-mediated insulin signaling cascade will probably provide a new strategy for therapeutic intervention in the treatment of type 2 diabetes and its complications (Dong et al., 2008; Guo, 2013). Interestingly, in the present study, the expression of the IRS-1 and -2 proteins in the hepatic tissue samples was significantly (P<0.05) lower in diabetic, untreated rats (Group III), when compared to untreated normal (Group I) rats. Naringenin treatment to diabetic (Group IV) rats, significantly (P<0.05) elevated the expression of IRS-1 and -2 proteins (Figure 1.4), suggesting that naringenin can increase IRS-mediated insulin signaling, thereby alleviating insulin resistance in hepatic tissues.

5. Conclusion

Administration of naringenin to rats with experimental diabetes mellitus significantly normalized the altered levels of glucose, glycosylated hemoglobin and urea in the blood, and serum insulin, AGE, creatinine and uric acid levels by virtue of its hypoglycemic property. Further, naringenin significantly normalized the altered levels of serum hepatic markers enzymes including AST, ALT, ALP and LDH and elevated the expression of IRS-1 and IRS-2 protein to improve IRS-mediated insulin signaling, so as to alleviate insulin resistance. The results suggest that naringenin confers protection against experimental diabetes by virtue of its anti-hyperglycemic effects possibly via stimulation of insulin secretion from the non-degraded pancreatic \( \beta \)-cells and also through alleviation of insulin resistance in hepatic tissues.
Chapter II

Modulatory effects of naringenin on carbohydrate and lipid metabolism in Wistar rats with streptozotocin-nicotinamide-induced diabetes mellitus

1. Introduction

Diabetes mellitus (diabetes) is a serious, complex chronic metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia and abnormalities in carbohydrate, protein, and lipid metabolism due to defects in insulin secretion or action, or both (American Diabetes Association, 2010). Chronic hyperglycemia in diabetes has a negative impact on various organs and tissues, such as pancreas, liver, kidneys, muscles and adipose tissues (Baynes, 1991; Maritim et al., 2003). Insulin regulates the carbohydrate metabolism by modulating the uptake and utilization of glucose in target organs such as the liver, kidneys, skeletal muscle and adipose tissue, by controlling the activities of numerous metabolic enzymes of glycolysis, gluconeogenesis and glycogen metabolism (Wilcox, 2005). Insulin resistance (an impaired insulin signaling), a key feature of impaired glucose tolerance in Type 2 diabetes mellitus, is characterized by diminished function of insulin-sensitive tissues and also by markedly-decreased glucose metabolism in response to insulin (DeFronzo and Tripathy, 2009). Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction (Wierzbicki, 1997). An increased level of circulating fatty acids and/or intracellular hepatic fat accumulation has recently been found to be involved in the pathogenesis of hepatic insulin resistance (Samuel and Shulman, 2012). Accumulation of intracellular lipid metabolites activate the serine kinase cascade involving protein kinase C (PKC), which, in turn, leads to decreased insulin receptor kinase activity and subsequent impaired insulin receptor substrate (IRS)-1 and IRS-2 tyrosine phosphorylation, leading to disruption of insulin signaling (Samuel et al., 2004). These