fat-induced defects in insulin signaling in turn cause in reduced insulin stimulation of glycogen synthase activity and reduced uptake and production of glucose by the liver (Saltiel and Kahn, 2001). Chronically elevated levels of glucose and fatty acids are hypothesized to adversely affect pancreatic β-cell function, thereby contributing to the deterioration of insulin secretion, irreversible changes in transcription of the genes encoding insulin production and β-cell mass with consequent low insulin production in Type 2 diabetes (Robertson et al., 2003; Scheen, 2003).

The results of the experiments described in the previous chapter suggested that naringenin protects against hyperglycemia in experimental diabetes possibly via stimulation of insulin secretion from the non-degraded pancreatic β-cells and also by increasing the IRS-mediated insulin signaling, thereby alleviating insulin resistance in hepatic tissue. The present chapter describes a detailed investigation that was performed to elucidate the modulatory influence of naringenin on IRS-mediated insulin signaling by studying key enzymes involved in carbohydrate and lipid metabolism as well as serum and hepatic lipid status.

2. Materials and methods

Animals, grouping of the experimental animals and treatment regimens used in this phase of the study were as described in the General Materials and Methods section (Pages 37 – 39).

2.1. Preparation of tissue homogenates

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

2.2.1. Assay of hexokinase

Hexokinase activity was assayed by the method of Brandstrup et al. (1957). The reaction mixture, in a total volume of 5.0 ml, contained: 1.0 ml of glucose solution, 0.5 ml of ATP solution, 0.1 ml of magnesium chloride, 0.4 ml of dipotassium hydrogen phosphate, 0.4 ml of potassium chloride, 0.1 ml of sodium fluoride and 2.5 ml of Tris-HCl buffer (pH 8.0); this was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of 2.0 ml of tissue homogenate. An aliquot (1.0 ml) of the reaction mixture was taken immediately (zero time) and transferred into tubes containing 1.0 ml of 10% trichloro acetic acid (TCA). A second aliquot of the reaction mixture was removed after 30 min of incubation at 37°C and likewise transferred to tubes containing 10% TCA. Precipitated protein was removed from the TCA-containing tubes by centrifugation and residual glucose in the supernatant was estimated by the O-toluidine method; a reagent blank was run with each test. The difference between the two values (Zero time ‘aliquot and 30 min ‘aliquot’) indicated the quantum of glucose phosphorylated. Hexokinase activity was expressed as μmoles of glucose-6-phosphate formed/h/mg of protein in the tissue homogenates under the conditions of incubation.

2.2.2. Assay of pyruvate kinase

Pyruvate kinase activity was assayed by the method of Pogson and Denton (1967). A incubation mixture containing 100 μl of Tris-HCl buffer, 240 μl of KCl, 50 μl of MgCl2, 10 μl of PEP, 2 μl of ADP, 0.5 μl of NADH, 100 μl of LDH and 20-40 μl of the enzyme source was incubated at 25°C for 5 min. The colour developed was read at 340 nm. The enzyme activity was expressed as μmoles of pyruvate formed/min/mg of protein in the tissue homogenates under conditions of incubation.
2.2.3. **Assay of Lactate dehydrogenase (LDH)**

LDH was assayed by the method of King (1965c), which is based on the conversion lactate to pyruvate by LDH in the presence of the coenzyme nicotinamide adenine dinucleotide (NAD+). The pyruvate formed was made to react with dinitrophenyl hydrazine (DNPH) in HCl, resulting in formation of hydrazone (an orange-coloured complex in alkaline medium), which was measured at 420 nm. The activity of LDH was expressed as mmoles of pyruvate formed/min/mg protein.

2.2.4. **Assay of Glucose-6-phosphate dehydrogenase (G6PDH)**

G6PDH was assayed by the method of Ells and Kirkman (1961), in which an incubation mixture containing 1 ml of Tris–HCl buffer (0.05 M, pH 7.5), 0.1 ml of magnesium chloride (0.1 M), 0.1 ml of NADP+ (0.1 M), 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and 0.2 ml of the enzyme extract was allowed to stand for 10 min at room temperature to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate, following which absorbance of the sample was read at 640 nm against blank (water) at 1 min intervals for 3–5 min. The enzyme was expressed in units by multiplying the change in OD/min by the factor 6/17.6, the molar extinction coefficient of the reduced co-enzyme.

2.2.5. **Assay of Glucose-6-phosphatase**

Glucose-6-phosphatase was assayed by the method of Koide and Oda (1959), in which on incubation mixture containing 0.7 ml of citrate buffer (0.1 M, pH 6.5), 0.3 ml of substrate (0.01M) and 0.3 ml of tissue homogenate was incubated at 37°C for 1 h. Addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant of tissue homogenate was estimated by the method of Fiske and Subbarow (1925). The supernatant was adjusted to a known volume, followed by addition of 1 ml of
ammonium molybdate and 0.4 ml of 1-amino, 2- naphthol, 4- sulfonic acid (ANSA). After 20 min the absorbance was read at 680 nm.

2.2.6. Assay of Fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphatase activity was measured by the method of Gancedo and Gancedo (1971) in which assay mixture (final volume 2 ml), containing 1.2 ml of Tris–HCl buffer (0.1 M, pH 7.0), 0.1 ml of substrate (0.05 M), 0.25 ml of magnesium chloride (0.1 M), 0.1 ml of potassium chloride solution (0.1 M), 0.25 ml of EDTA (0.001M) solution and 0.1 ml of enzyme homogenate, was incubated at 37°C for 5 min following which the reaction was terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant used for phosphorus determination by the method of Fiske and Subbarow (1925), as described above. The activities of glucose-6-phosphatase and fructose-1,6- bisphosphatase were expressed as μmol of Pi liberated/min.

2.3. Determination of glycogen metabolism in hepatic tissues

2.3.1. Estimation of glycogen concentrations in hepatic tissues

For the estimation of glycogen, alkali extraction was first performed as described by Morales et al. (1973). Glycogen was precipitated from the alkali extract of the hepatic tissues by adding 1:3 volume of 95% ethanol and a drop of 1 M ammonium acetate. The tubes were kept in a boiling water bath for 5 min. After cooling, the tubes were shaken well and placed in a freezer overnight. The precipitated glycogen was then collected by centrifugation at 3,000 x g for 40 min. The precipitate was dissolved in water, precipitated with alcohol and centrifuged again. The final precipitate was dissolved in 3.0 ml of water and heated for 5 min in a boiling water bath and this extract was used for the estimation of glycogen. 0.5 ml of this extract was made up to 1.0 ml with water. A set of standard glucose solutions (25-100 μg) and blank containing water alone were set up. All the tubes were cooled in an ice-bath and 4.0 ml of anthrone
reagent was added. The contents of the tubes were mixed well. All the tubes were covered with glass marbles and heated for 20 min in a boiling water bath. The tubes were cooled and the green colour developed was read at 640 nm using a spectrophotometer. Values of glycogen were expressed as mg of glucose/g of wet hepatic tissue.

### 2.3.2. Assay of glycogen synthase in hepatic tissues

Glycogen synthase activity was assayed by the method of Leloir and Goldenberg (Leloir and Goldenberg, 1962). The following components were mixed: 0.09 ml each of glycogen, buffer, glucose-6-phosphate solution, 0.015 ml of cysteine hydrochloride solution and 0.5 ml of enzyme source. The reaction was started by the addition of 0.03 ml of uridine diphosphate (UDP) glucose and the incubation was performed at 37°C for 5-10 min. The tubes were then heated in a boiling water bath for one min. A blank in which UDP glucose was added after incubation was run at the same time with the UDP standards (10-60 μg). To estimate the UDP formed in the reaction, 0.075 ml each of phosphoenol pyruvate solution and pyruvate kinase were added. The tubes were incubated for 15 min at 37°C and 0.45 ml of dinitrophenyl hydrazine solution was then added. After 10 min, 0.60 ml of 10 N sodium hydroxide solutions and 1.1 ml of ethanol were added, the contents were mixed well and centrifuged. The optical density of the supernatant fluid was read at 530 nm using spectrophotometer. The enzyme activity was expressed as μmoles of UDP formed/h/mg of protein.

### 2.3.3. Assay of glycogen phosphorylase in hepatic tissues

Glycogen phosphorylase activity was assayed by the method of Cornblath et al. (1963). The reaction was started by the addition of 1.0 ml of substrate, 0.1 ml of 5′ AMP, 0.2 ml of sodium fluoride and 0.2 ml of enzyme. An aliquot (0.5 ml) was taken from the reaction mixture at zero time and, after 10 min of incubation at 37°C,
1.0 ml of 10% TCA solution was added to arrest the reaction. The contents were mixed well, centrifuged and the liberated phosphorus in the supernatant was estimated by the method of Fiske and Subbarow (1925). The enzyme activity was expressed as μmoles of phosphate liberated/h/mg of protein.

2.4. Assay of lipoprotein lipase (LPL) in hepatic tissue samples

The activity of LPL was assayed by the method of Baginsky (1981). The colour developed was read spectrophotometrically at 430 nm. The assay was standardized against a glycerol solution of known molarity. Activity of LPL was expressed as μmoles of glycerol liberated/h/mg of protein.

2.5. Assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase) in hepatic tissue sample

The ratio between HMG CoA and mevalonate in the hepatic tissue was taken as an index of the activity of HMG CoA-reductase (Rao and Ramakrishnan, 1975). Equal volumes of fresh 10% hepatic tissue homogenate and diluted perchloric acid (5%) were mixed, kept for 5 min and centrifuged at 3000 rpm for 10 min. To 1.0 ml of filtrate, 0.5 ml of freshly prepared 50% hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG CoA) was added and mixed. After 5 min, 1.5 ml of ferric chloride were added and then shaken well. Readings were taken after 10 min at 540 nm against a similarly-treated saline-arsenate (0.1%) blank. The ratio of HMG CoA to mevalonate was calculated; a low ratio was considered to indicate high enzyme activity and vice-versa.

2.6. Extraction of hepatic and renal tissue lipids

Hepatic tissue lipids were extracted by the method of Folch et al. (1957). Briefly, the tissue was homogenized with chloroform/methanol solvent mixture (2:1, v/v) and the volume of solvent mixture was adjusted to 20 times the weight of the
hepatic tissue sample (1 g in 20 ml of solvent mixture). After dispersion, the entire mixture was agitated for 15-20 min in an orbital shaker at room temperature. The homogenate was filtered through Whatmann No.1 filter paper into a conical flask. The solvent was washed with 0.9% NaCl solution. After vortexing for a few seconds, the mixture was centrifuged at 2000 rpm for 5 minutes to separate the two phases. The upper aqueous phase containing gangliosides and other water-soluble compounds was removed. The lower chloroform phase containing lipids was evaporated to dryness and the lipid extract was then used for the estimation of fatty acids.

2.6.1. Estimation of Total cholesterol (TC)

Total cholesterol content was estimated by the method of Parekh and Jung (1970). In brief, approximately 0.1 ml of test sample was made up to 10 ml with ferric acetate-uranyl acetate reagent. 0.1 ml of an aliquot of the total lipid extract was evaporated to dryness; this dried extract and standards were made up to 3 ml with ferric chloride-uranyl acetate reagent. Then, 2.0 ml of sulphuric acid-ferrous sulphate reagent were added to all the tubes and the contents were mixed well. After 20 min, the colour developed was read at 540 nm using a UV spectrophotometer. The levels were expressed as mg/g of fresh tissue.

2.6.2. Estimation of triglycerides (TG)

TG was estimated by the method of Rice (1970). Four millilitres of the lipid extract were added to tubes containing 8.0 ml of saturated sodium chloride and shaken vigorously. The contents were allowed to settle for 1 h and then centrifuged. The supernatant of the saline-methanol phase were discarded. The washed chloroform phase was filtered into a dry tube, 200 mg of activated silicic acid were added to the chloroform phase, shaken vigorously and allowed to stand for 30 min. After centrifugation, 0.5 ml of the supernatant, as well as tripalmitin standards were evaporated to dryness. Then to the test, standard and blank tubes, 0.5 ml of alcoholic
potassium hydroxide solution was added and the mixture was saponified in a 60-70°C water bath for 20 min; following this, 0.5 ml of 0.2 N sulphuric acid was added and heated in a boiling water bath for 10 min. After cooling the tubes, 0.1 ml of sodium metaperiodate was added and allowed to stand for 10 min. The excess periodate was reduced by the addition of 0.1 ml of sodium arsenite. Following this, 5.0 ml of chromotropic acid reagent was added, mixed thoroughly and kept in a boiling water bath for 30 min. After cooling, 0.5 ml of thiourea solution was added. The colour developed was read at 540 nm against a blank using a spectrophotometer. Concentration of TG was expressed as mg/dl in plasma and mg/g in fresh tissue.

2.6.3. Estimation of phospholipids

Phospholipid concentration was estimated by digestion with perchloric acid (Bartlett, 1959) and the phosphate liberated was estimated (Fiske and Subbarow, 1925). In brief, various aliquots of the test sample (each 0.2 ml) were digested with 0.5 ml of perchloric acid over a sand-bath until the sample became colorless and clear. A standard solution of phosphorous (8 to 32 μg) as well as a blank was mixed with perchloric acid and treated in a similar manner. The volume in all the tubes was made up to 4.3 ml with double distilled water, following which first ammonium molybdate (0.5 ml) and then ANSA reagent (0.2 ml) were added. The blue colour developed was read after 20 min at 640 nm. Phosphorus content was multiplied by a factor of 25, which yielded the concentration of phospholipids in the test samples.

2.7. Determination of lipid profile in serum

Various parameters of the serum lipid profile, including the levels of TC, TG, low density lipoprotein (LDL)-cholesterol, very low density lipoprotein (VLDL)-cholesterol and high density lipoprotein (HDL)-cholesterol were determined by using standard assay kits (Diasys, Germany). The units for each parameter were expressed as mg/dl.
2.8. 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. Activities of glycolytic enzymes in Wistar rats

Tables 2.1 and 2.2 list the mean activities of hexokinase and pyruvate kinase enzymes in hepatic and renal tissue samples from each of the five groups of rats. There were no significant differences between the mean activities of these enzymes in the hepatic and renal tissue samples from normal naringenin-treated (Group II) rats and of normal untreated (Group I) rats. Significantly (P<0.05) lower mean activities of these glycolytic enzymes were observed in hepatic and renal tissue samples from diabetic untreated (Group III) rats than those from Group I and Group II rats. However, significantly (P<0.05) higher mean activities of these enzymes were observed in hepatic and renal tissue samples from diabetic rats treated with naringenin (Group IV) and diabetic rats treated with glyclazide (Group V) than those from Group III rats. Interestingly, there were no significant differences in the mean values of these parameters between samples from naringenin-treated diabetic (Group IV) rats and glyclazide-treated diabetic (Group V) rats. Although significantly higher mean enzyme activities were noted in samples from Group IV and Group V rats (compared to the levels in samples from diabetic untreated rats), the values still differed significantly
from those observed in hepatic and renal tissue samples from normal untreated (Group I) and normal naringenin-treated (Group II) rats.

3.2. Activities of G6PDH and LDH enzymes in hepatic and renal tissue samples of Wistar rats

Tables 2.1 and 2.2 list the mean activities of G6PDH and LDH enzymes in hepatic and renal tissue samples from each of the five groups of rats. Samples from Group II rats did not exhibit significant differences in the test parameters when compared to those from Group I rats. Hepatic and renal tissue samples from Group III rats showed significantly (P<0.05) lower mean activity of G6PDH and significantly (P<0.05) higher mean activity of LDH, when compared to values in samples from Group I and Group II rats. Conversely, hepatic and renal tissue samples from Group IV and Group V rats exhibited significantly (P<0.05) higher mean activities of G6PDH and significantly (P<0.05) lower mean activities of LDH, when compared to the values in hepatic and renal tissue samples from Group III rats. However, although there were significant improvements in these parameters in samples from Group IV and Group V rats (compared to the levels in samples from diabetic untreated rats), the mean values in samples from Group IV and Group V rats still differed significantly from those observed in the samples from Group I and Group II rats.

3.3. Activities of gluconeogenic enzymes in Wistar rats

There were no significant differences between the mean activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase enzymes in hepatic and renal tissue samples from Group I and Group II rats. The mean activities of these gluconeogenic enzymes in hepatic and renal tissue samples from Group III rats were significantly higher than those in samples from normal untreated (Group I) and normal naringenin-treated (Group II) rats (Tables 2.1 & 2.2). However, significantly (P<0.05) lower mean activities of these enzymes were observed in samples Group IV and Group V rats than
### Table 2.1. Mean activities of enzymes of carbohydrate metabolism in hepatic tissues of Wistar rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Group I (normal untreated)</th>
<th>Group II (normal naringenin-treated)</th>
<th>Group III (diabetic untreated)</th>
<th>Group IV (diabetic naringenin-treated)</th>
<th>Group V (diabetic glyclazide-treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>269.67 ± 7.69</td>
<td>258.67 ± 7.69</td>
<td>131.67 ± 5.72a</td>
<td>215.33 ± 8.28ab</td>
<td>223.33 ± 7.24ab</td>
</tr>
<tr>
<td>Pyruvatekinase</td>
<td>213.05 ± 7.72</td>
<td>210.83 ± 9.98</td>
<td>96.67 ± 4.62a</td>
<td>165.21 ± 5.21ab</td>
<td>170.55 ± 7.1ab</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>242.50 ± 10.57</td>
<td>239.50 ± 12.17</td>
<td>494.33 ± 14.38a</td>
<td>282.33 ± 16.71ab</td>
<td>273.50 ± 12.56ab</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>1106.17 ± 49.57</td>
<td>1174.17 ± 43.28</td>
<td>2238.50 ± 84.17a</td>
<td>1308.33 ± 47.49ab</td>
<td>1287.67 ± 50.5a</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
<td>492.50 ± 14.77</td>
<td>495.83 ± 12.32</td>
<td>817.33 ± 13.07a</td>
<td>543.50 ± 13.59ab</td>
<td>536.31 ± 12.4a</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>512.31 ± 10.97</td>
<td>517.67 ± 13.91</td>
<td>263.33 ± 7.86a</td>
<td>405.04 ± 8.85ab</td>
<td>412.67 ± 9.21ab</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]

Units are expressed as: μ moles of glucose-6-phosphate formed/h/mg protein for hexokinase; mU/mg of protein for pyruvate kinase; μmoles of pyruvate formed/h/mg protein for lactate dehydrogenase; μmoles of Pi liberated/h/mg protein for glucose-6-phosphatase and fructose-1,6-bisphosphatase; μmoles of NADPH/min/mg protein for glucose-6-phosphate dehydrogenase.

*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 2.2. Mean activities of enzymes of carbohydrate metabolism in renal tissues of Wistar rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Group I (normal untreated)</th>
<th>Group II (normal naringenin-treated)</th>
<th>Group III (diabetic untreated)</th>
<th>Group IV (diabetic naringenin-treated)</th>
<th>Group V (diabetic glyclazide-treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>163.33 ± 3.83</td>
<td>160.17 ± 8.42</td>
<td>95.66 ± 2.70</td>
<td>124.76 ± 5.69 a,b</td>
<td>131.33 ± 7.43 a,b</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>162.83 ± 5.05</td>
<td>153.31 ± 4.62</td>
<td>81.17 ± 3.68 a</td>
<td>132.74 ± 1.79 a,b,c</td>
<td>138.09 ± 4.0 a,b</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>494.50 ± 8.09</td>
<td>511.6 ± 12.26</td>
<td>739.50 ± 13.94 a</td>
<td>556.17 ± 9.38 a,b,c</td>
<td>529.67 ± 8.64 a,b</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>439.17 ± 1029</td>
<td>434.54 ± 13.98</td>
<td>635.43 ± 10.72 a</td>
<td>513.67 ± 12.57 a,b</td>
<td>504.13 ± 10.08 a,b</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
<td>749.52 ± 19.21</td>
<td>752.49 ± 20.97</td>
<td>952.03 ± 14024 a</td>
<td>813.74 ± 18.93 a,b</td>
<td>804.17 ± 18.93 a,b</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>614.17 ± 10.83</td>
<td>590.66 ± 10.48</td>
<td>292.51 ± 9.78 a</td>
<td>490.33 ± 6.84 a,b,c</td>
<td>514.83 ± 11.35 a,b</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]

Units are expressed as: µ moles of glucose-6-phosphate formed/h/mg protein for hexokinase; mU/mg of protein for pyruvate kinase; µmoles of pyruvate formed/h/mg protein for lactate dehydrogenase; µmoles of Pi liberated/h/mg protein for glucose-6-phosphatase and fructose-1,6-bisphosphatase; µmoles of NADPH/min/mg protein for glucose-6-phosphate dehydrogenase.

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.
those in samples from Group III rats, although the levels remained higher than those in samples from Group I and Group II rats (Tables 2.1 & 2.2). Interestingly, there were no significant differences between the mean values of these parameters in hepatic and renal tissue samples from Group IV and Group V rats (Tables 2.1 & 2.2).

3.4. Mean level of glycogen and activities of enzymes of glycogen metabolism in hepatic tissues of Wistar rats

Table 2.3 lists the mean level of glycogen and the mean activities of glycogen synthase and glycogen phosphorylase enzymes in hepatic tissue samples from each of the five groups of rats. Samples from Group II did not exhibit significant differences in the test parameters when compared to samples from Group I rats. Group III rats showed significantly (P<0.05) lower mean hepatic levels of glycogen and mean hepatic activities of glycogen synthase and significantly (P<0.05) higher mean activity of glycogen phosphorylase, when compared to mean levels/activities in hepatic tissue samples from Group I and Group II rats. Conversely, Group IV and Group V rats exhibited significantly (P<0.05) higher mean hepatic levels of glycogen and mean hepatic activities of glycogen synthase and significantly (P<0.05) lower mean activity of glycogen phosphorylase, when compared to mean levels/activities in hepatic tissue samples from Group III rats. However, although mean levels/activities of these parameters in hepatic tissue samples from Group IV and Group V rats were significantly better than those in samples from diabetic untreated rats, they still differed significantly from those observed in the Group I and Group II rat hepatic tissue samples.

3.5. Activities of enzymes of cholesterol metabolism in hepatic tissues of Wistar rats

Significantly (P<0.05) lower mean activities of LPL and significantly (P<0.05) higher mean activities of HMG CoA-reductase were noted in hepatic tissues of Group
### Table 2.3. Mean level of glycogen and activities of enzymes of glycogen metabolism in hepatic tissues of Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Glycogen (mg/g wet tissue)</th>
<th>Glycogen synthase ($\mu$moles of UDP formed/h/mg protein)</th>
<th>Glycogen phosphorylase ($\mu$moles Pi liberated /h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>$60.83 \pm 1.35$</td>
<td>$842.17 \pm 10.91$</td>
<td>$658.33 \pm 11.46$</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>$56.67 \pm 1.43$</td>
<td>$830.33 \pm 16.96$</td>
<td>$647.83 \pm 12.42$</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>$19.50 \pm 2.35^a$</td>
<td>$525.67 \pm 14.39^a$</td>
<td>$897.50 \pm 28.26^a$</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>$44.83 \pm 1.14^{a,b,c}$</td>
<td>$760.17 \pm 10.88^{a,b,c}$</td>
<td>$744.17 \pm 16.03^{a,b,c}$</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>$49.00 \pm 2.24^{a,b}$</td>
<td>$791.50 \pm 12.25^{a,b}$</td>
<td>$724.33 \pm 14.81^{a,b}$</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
- $^c$ Statistically significant difference (P<0.05) when compared with Group V values
III rats, when compared to values in hepatic tissue samples from Group I and Group II rats (Table 2.4). However, significantly (P<0.05) higher mean activities of LPL and significantly (P<0.05) lower mean activities of HMG CoA-reductase were observed in hepatic tissue samples from Group IV and Group V rats than those in Group III rats (Table 2.4). However, although the mean activities of these enzymes were significantly better in hepatic tissue samples from Group IV and Group V rats than those in hepatic tissue samples from diabetic untreated (Group III) rats, they were still significantly different from the values noted in samples from Group I and Group II rats (Table 2.4).

3.6. **Mean level of TC, TG and phospholipids in hepatic and renal tissues**

Tables 2.5 and 2.6 list the mean levels of TC, TG and phospholipids in hepatic and renal tissue samples from each of the five groups of rats. Group II did not exhibit significant differences in the test parameters when compared to Group I rats. In Group III rats, TC, TG and phospholipid levels in hepatic and renal tissues were significantly (P<0.05) higher than those in Group I and Group II rat tissue samples. However, significantly (P<0.05) lower mean levels of these parameters were observed in hepatic and renal tissue samples from Group IV and Group V rats than those in samples from Group III rats. The mean levels of these parameters in samples from Group IV and Group V rats were significantly higher than those in Group I and Group II rat samples (Tables 2.5 & 2.6).

3.7. **Mean level of serum lipid profile parameters**

Normal naringenin-treated (Group II) rats did not exhibit significant differences in mean levels of serum lipid profile parameters when compared to values in normal untreated (Group I) rats (Table 2.7). In diabetic untreated (Group III) rats, serum TC, TG, LDL and VLDL cholesterol levels were significantly (P<0.05) higher and serum HDL cholesterol level was significantly (P<0.05) lower than those in serum samples from Group I and Group II rats (Table 2.7). In diabetic rats that had been treated with
Table 2.4. Mean activities of lipid-metabolizing enzyme and rate limiting enzyme of cholesterol biosynthesis in hepatic tissues of Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Lipoprotein lipase (µmoles of glycerol liberated/h/mg protein)</th>
<th>HMG CoA reductase (ratio of HMG CoA to mevalonate)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>13.85 ± 0.9</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>13.12 ± 0.7</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>9.79 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>11.56 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.1 ± 0.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>12.51 ± 0.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.3 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Lower ratio indicates higher enzyme activity and vice versa

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

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

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

- Statistically significant difference ($P<0.05$) when compared with Group III values.
Table 2.5. Mean levels of total cholesterol, triglyceride and phospholipid in hepatic tissues of Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>7.58 ± 0.9</td>
<td>3.5 ± 0.3</td>
<td>25.63 ± 1.9</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>7.21 ± 0.7</td>
<td>3.7 ± 0.9</td>
<td>26.92 ± 1.3</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>15.97 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.97 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>9.65 ± 0.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.6 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>29.84 ± 1.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>8.5 ± 0.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.0 ± 0.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>28.85 ± 1.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

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

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

<sup>b</sup> Statistically significant difference (P<0.05) when compared with Group III values
Table 2.6. Mean levels of total cholesterol, triglycerides and phospholipids in renal tissues of Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal untreated)</td>
<td>4.85 ± 0.9</td>
<td>2.8 ± 0.4</td>
<td>16.63 ± 1.7</td>
</tr>
<tr>
<td>Group II (naringenin-treated)</td>
<td>4.12 ± 0.7</td>
<td>2.7 ± 0.7</td>
<td>15.92 ± 1.3</td>
</tr>
<tr>
<td>Group III (diabetic untreated)</td>
<td>15.79 ± 0.3(^a)</td>
<td>6.4 ± 0.2(^a)</td>
<td>27.97 ± 2.1(^a)</td>
</tr>
<tr>
<td>Group IV (diabetic naringenin-treated)</td>
<td>7.56 ± 0.8(^a,b)</td>
<td>3.1 ± 0.7(^a,b)</td>
<td>19.84 ± 1.5(^a,b)</td>
</tr>
<tr>
<td>Group V (diabetic glyclazide-treated)</td>
<td>6.51 ± 0.9(^a,b)</td>
<td>3.0 ± 0.2(^a,b)</td>
<td>18.85 ± 1.4(^a,b)</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD for observations 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 2.7. Mean activities of serum lipid profile parameters in Wistar rats

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Group I (normal untreated)</th>
<th>Group II (normal naringenin-treated)</th>
<th>Group III (diabetic untreated)</th>
<th>Group IV (diabetic naringenin-treated)</th>
<th>Group V (diabetic glyclazide-treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>51.23 ± 4.98</td>
<td>54.41 ± 2.81</td>
<td>155.94 ± 5.76 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.22 ± 6.81 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>69.23 ± 7.32 &lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>49.59 ± 4.25</td>
<td>52.52 ± 7.93</td>
<td>136.75 ± 2.34 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.34 ± 4.15 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>60.51 ± 2.76 &lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>33.17 ± 3.82</td>
<td>32.04 ± 1.98</td>
<td>17.42 ± 2.11 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.29 ± 2.79 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>25.21 ± 1.01 &lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>13.19 ± 2.1</td>
<td>12.39 ± 1.17</td>
<td>111.1 ± 3.63 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.87 ± 5.06 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>31.92 ± 2.56 &lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>9.91 ± 0.85</td>
<td>8.55 ± 0.79</td>
<td>27.35 ± 0.16 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.06 ± 0.48 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>12.10 ± 1.00 &lt;sup&gt;a,b&lt;/sup&gt;</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]

Abbreviations: TC= Total Cholesterol; TG= triglycerides; HDL-C = High-density lipoprotein-cholesterol; LDL-C = Low-density lipoprotein-cholesterol; VLDL-C = Very low-density lipoprotein-cholesterol;

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

<sup>b</sup> Statistically significant difference (P<0.05) when compared with Group III values
naringenin (Group IV), such alterations did not occur, and values were maintained at near normal levels and approximated those in diabetic rats treated with glyclazide (Group V) rats; however, the mean serum levels of these test parameters in Group IV and Group V rats were significantly lower than those in Group I and Group II rats (Table 2.7).

4. Discussion

The liver is an important organ that plays a central role in maintaining glucose homeostasis through glycolysis and gluconeogenesis (Roden and Bernroider, 2003; Gastaldelli et al., 2004), with a minor contribution from the kidney (Cersosimo et al., 1997). Diabetes mellitus is characterized by a reduced capacity of the $\beta$-cells of the pancreas to release sufficient insulin to induce the activity of glucose-metabolizing enzymes (Panneerselvam and Govindaswamy, 2002). A partial or total deficiency of insulin, or resistance to insulin action, causes derangement in carbohydrate metabolism that decreases the activity of several key enzymes of glycolysis including hexokinase, pyruvate kinase and lactate dehydrogenase and increases the activities of gluconeogenic enzymes including glucose-6-phosphatase and fructose 1,6-bis-phosphatase, resulting in impaired peripheral glucose utilization and augmented hepatic glucose production, respectively (Wilcox, 2005).

One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it to glucose-6-phosphate (Wilson, 1995). Pyruvate kinase is a ubiquitously expressed, rate-controlling, terminal, key glycolytic enzyme that catalyzes the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP (Yamada and Noguchi, 1999). In the present study, the observed lower mean activity of hexokinase in hepatic and renal tissues of diabetic untreated rats was possibly due to the decreased levels of insulin and/or increased insulin resistance in the streptozotocin (STZ)-nicotinamide administered rats, since insulin deficiency and
resistance constitute the hallmark of diabetes (Ferrannini, 1998). However, the mean activity of hexokinase in naringenin-treated diabetic (Group IV) rats (Tables 2.1 & 2.2) were significantly higher than that in Group III rats, suggesting that naringenin may augment the activity of hexokinase that leads to enhanced glucose metabolism and promotes overall glucose homeostasis.

LDH is a terminal glycolytic enzyme that plays an indispensable role in the inter-conversion of pyruvate to lactate to yield energy under anaerobic conditions (Kavanagh \textit{et al.}, 2004). The decreased activity of LDH in tissues may be important in ensuring that a high proportion of both pyruvate and NADH, supplied by glycolysis, is subsequently oxidized by mitochondria. Indeed, elevated LDH levels observed in experimental diabetic animals are associated with impaired glucose-stimulated insulin secretion (Ainscow \textit{et al.}, 2000). In the present investigation, the mean activities of LDH in hepatic and renal tissues were significantly (P<0.05) higher in diabetic untreated (Group III) rats than those in normal (Group I and Group II) rats. However, naringenin-treated diabetic rats, the mean LDH activity in hepatic and renal tissues was significantly (P<0.05) lower than that in hepatic and renal tissues of diabetic untreated rats (Tables 2.1 & 2.2). These results suggest that naringenin may alleviate the impaired glucose-stimulated insulin secretion and augment its action, thereby enhancing glucose utilization and maintaining glucose homeostasis.

G6PDH, the first and rate-limiting enzyme of the pentose phosphate pathway, produces reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is needed for the maintenance of reduced glutathione and reductive biosynthesis (Wagle \textit{et al.}, 1998). In the present investigation, the activity of G6PDH in diabetic untreated rats was significantly lower than that in normal untreated rats (Tables 2.1 & 2.2). Other investigators have also reported decreased activity of G6PDH in the diabetic state (Panneerselvam and Govindaswamy, 2002; Zhang \textit{et al.}, 2010). Decreased activity of
G6PDH in diabetes may slow down the pentose phosphate pathway, thereby lowering the production of the reducing agent NADPH and inducing oxidative stress (Ugochukwu and Babady, 2002), finally resulting in diabetic complications (Zhang et al., 2010). Interestingly, in the present study, naringenin-treated diabetic rats exhibited significantly (P<0.05) higher mean activity of G6PDH in hepatic and renal tissues when compared to values noted in diabetic, untreated rats (Tables 2.1 & 2.2). Naringenin possibly acts by increasing the influx of glucose into the pentose monophosphate shunt by inducing increased secretion of insulin, leading to elevation of NADPH that may avert or retard induction of oxidative stress. Ugochukwu et al. (2003) reported a similar phenomenon in rats with STZ-induced diabetes mellitus that had been treated with an extract of Gongronema latifolium.

Glucose-6-phosphatase, a key terminal enzyme of gluconeogenesis and glycogenolysis, catalyzes the dephosphorylation of glucose-6-phosphate to free glucose (Bouche et al., 2004). Fructose-1,6-bisphosphatase is a highly-regulated, rate-limiting enzyme, which catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate, the second to last step in the gluconeogenic pathway (Pilkis and Claus, 1991). Van de Werve et al. (2000) have demonstrated that the increased rate of gluconeogenesis in diabetes mellitus is related to the increased expression of key gluconeogenic enzymes in hepatic tissues. Hence, studying the effect of naringenin on gluconeogenesis may further elucidate its putative hypoglycemic action. Interestingly, in the present study, the activities of glucose 6-phosphatase and fructose-1,6-bisphosphatase were significantly decreased in diabetic untreated (Group III) rats when compared to normal untreated (Group I) and normal naringenin-treated (Group II) rats (Tables 2.1 & 2.2). However, in naringenin-treated diabetic (Group IV) rats, significantly (P<0.05) higher mean activities of G6PDH in hepatic and renal tissues were noted when compared to the values in diabetic, untreated rats (Tables 2.1 & 2.2).
These results are consistent with those of Pari and Sankaranarayanan (2009), who reported that thymoquinone, an active principle of the volatile oil of black cumin seeds, reduces hepatic glucose production through gluconeogenesis. Naringenin possibly inhibits the key enzymes of gluconeogenesis in response to increased secretion or action of insulin, thereby decreasing the endogenous glucose production that occurs in gluconeogenesis.

Glycogen is the primary storable form of intracellular glucose and its levels in various tissues, especially liver and skeletal muscle, are a direct reflection of insulin activity, since insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Golden et al., 1979). Glycogen synthase, a crucial and rate-limiting enzyme in tissues with non-oxidative glucose disposal, catalyzes the transfer of glucose from UDP-glucose to glycogen in animal cells; its activity is regulated by decreased cellular glycogen content, hormone signaling, subcellular localization and allosteric activation by glucose-6-phosphate (Parker et al., 2004). Glycogen phosphorylase, also a rate-limiting enzyme of glycogenolysis, cleaves the $\alpha \ (1\rightarrow4)$ linkage to remove glucose molecules from glycogen (Greenberg et al., 2006); its activity is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose (Bollen et al., 1998). Hepatic glycogen content is markedly decreased in diabetic animals (Steiner and King, 1964; Weber and Singhal, 1964), in proportion to insulin deficiency (Stalmans et al., 1997). Interestingly, in the present investigation significantly lower mean activity of glycogen synthase and significantly lower mean level of glycogen and significantly (P<0.05) higher mean activity of glycogen phosphorylase were noted in hepatic tissues in diabetic untreated (Group III) rats, when compared to activities/levels in normal (Group I and Group II) rats (Table 2.3). However, administration of naringenin to rats with STZ-nicotinamide-induced diabetes mellitus appeared to maintain the level of
glycogen and the activity of glycogen synthase and phosphorylase in hepatic tissues at near normal levels (Table 2.3). Oral administration of naringenin to diabetic rats possibly regulated the activity of glycogen-metabolizing enzymes by stimulating the remaining pancreatic β-cells to secrete additional quantities of insulin thereby normalizing the altered glycogen content. Recently, Palsamy and Subramanian, (2009) reported a similar phenomenon in rats with STZ-nicotinamide-induced diabetes mellitus that had been treated with resveratrol, a natural phytoalexin.

The liver is considered to be one of the central metabolic organs of the body, regulating and maintaining lipid homeostasis. Further, it has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes which, in turn, leads to accumulation of lipids in hepatic tissue (Thilakarathna et al., 2013). The most commonly observed lipid abnormality in diabetes is hypercholesterolemia. The degree of hypercholesterolemia is directly proportional to the severity of diabetes (Shepherd, 2005). HMG CoA-reductase, the rate-limiting enzyme in cholesterol biosynthesis, catalyzes the conversion of HMG CoA to mevalonate using NADPH as a reducing equivalent. LPL, a lipid-metabolizing enzyme, plays an important role in the metabolism of plasma lipoproteins and, thus, the transport of lipids to peripheral tissues. Reduced activity of LPL leads to marked hyperlipidemia and hypertriglyceridemia (Bensadoun, 1991; Reymer et al., 1995). Interestingly, in the present investigation, the mean activity of HMG CoA-reductase was significantly (P<0.05) higher and the mean activity of LPL was significantly lower in hepatic tissue samples from diabetic untreated (Group III) rats than the values noted in normal untreated (Group I) and normal naringenin-treated (Group II) rats (Table 2.4). Further, mean TC, TG and phospholipid concentrations were significantly higher in hepatic and renal tissues of diabetic untreated rats when compared to values in normal (Group I and Group II) rats (Tables 2.5 & 2.6). However, in naringenin-treated
Diabetic (Group IV) rats, significantly higher mean activities of LPL and significantly lower mean activities of HMG CoA-reductase, as well as significantly lower mean levels of TC, TG and phospholipids, were noted, when compared to the values in diabetic untreated (Group III) rats (Tables 2.4, 2.5 & 2.6).

Diabetes mellitus is also associated with profound alterations in the serum lipoprotein profile and with an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction (Wierzbicki, 1997). High levels of total cholesterol and, more importantly, LDL cholesterol, in blood are major coronary risk factors (Wilson et al., 1998). Further, Wilson, (1990) reported that an increase in HDL cholesterol is associated with a decrease in coronary risk, but most antihyperlipidemic drugs appear to cause significant reductions in HDL cholesterol levels. Insulin deficiency is associated with abnormally high concentrations of serum lipids; this is mainly due to an increase in mobilization of free fatty acids from the peripheral fat depots, which tends to promote synthesis of phospholipids and cholesteryl esters by the liver and kidney (Howard, 1987). Santomauro (1999) reported that a reduction in circulating free fatty acid and its oxidation would be expected to improve hyperglycemia and strengthen the insulin response through increasing the hepatic gluconeogenesis and reducing glucose utilization. In the present study, mean serum levels of TC, LDL-cholesterol, VLDL- cholesterol and TG were significantly higher and mean serum HDL-cholesterol levels were significantly lower in diabetic untreated (Group III) rats when compared to levels in normal (Group I and Group II) rats (Table 2.7). However, in naringenin-treated diabetic rats, such alterations were not observed, and the values approximated near normal levels and those in glyclazide-treated rats (Table 2.7). Naringenin may prevent serum dyslipidemia and accumulation of lipids in hepatic and renal tissues by amelioration of abnormalities in hepatic cholesterol metabolism and by alleviating insulin resistance.
5. Conclusion

The results of the present study strongly suggest that naringenin confers protection against experimental STZ-nicotinamide-induced diabetes mellitus in Wistar rats by virtue of antihyperglycemic and antihyperlipidemic effects mediated through decreased insulin deficiency and decreased insulin resistance. Naringenin appears to exert its antihyperglycemic and anti-hyperlipidemic action through several mechanisms: by normalizing the disturbed carbohydrate metabolism via enhancing glucose utilization through increased glycolysis and glycogen synthesis and decreasing hepatic glucose production through gluconeogenesis and glycogenolysis; by replenishing the lipid metabolizing enzymes and rate-limiting enzymes of cholesterol biosynthesis; by correcting abnormalities in the serum lipid profile and hepatic and renal TC, TG and phospholipids levels.
Chapter III

Anti-oxidative potential of naringenin in Wistar rats with streptozotocin-nicotinamide-induced diabetes mellitus

1. Introduction

The results of investigations in experimental animal models of diabetes and in human diabetic subjects have suggested that diabetes mellitus is associated with a state of enhanced oxidative stress, resulting from the combination of increased generation of reactive free radicals and decreased antioxidant capacity, particularly during poor glycemic control (Altomare, 1992; Sundaram et al., 1996; Bonnefont-Rousselot et al., 2000; West, 2000; Maritim et al., 2003). Chronic hyperglycemia in diabetes leads to auto-oxidation of glucose, non-enzymatic protein glycosylation, impaired glutathione metabolism, alterations in antioxidant enzymes and formation of lipid peroxides; these events accelerate production of free radicals and weaken the antioxidant defense (Mullarkey et al., 1990; McLennan et al., 1991; Strain, 1991; Bagri et al., 2009). Uncontrolled production of free radicals or inadequate efficacy of antioxidant defense mechanisms can lead to damage of cellular structures and enzymes (Flora, 2007).

Increased production of reactive oxygen species may play a major role in the destruction of the pancreas and the progression of β-cell dysfunction (Kajimoto and Kaneto, 2004). Pancreatic β-cells are highly prone to oxidative stress and damage because of relatively low expression of antioxidant enzymes by and activities of antioxidant enzymes, which are the first line of defense against oxidative insult (Eizirik et al., 1994; Lenzen et al., 1996). In addition to the pancreatic β-cells, supraphysiological glucose levels are notorious in provoking oxidative stress particularly in hepatic and renal tissues, leading to damage in these tissues. Furthermore, oxidative stress is postulated to be the main metabolic abnormality...