Chapter 9

A COMBINATION OF EXERCISE AND MELATONIN SUPPLEMENTATION EXERTS COMPLEMENTING SYNERGISTIC EFFECT IN REVERSING DIABETES INDUCED DISTURBANCE IN GLUCOSE METABOLISM, DYSLIPIDEMIA AND OXIDATIVE STRESS

Diabetes is a universal metabolic disorder, with an increasing global trend and of particular concern to India, which is fast developing into the world capital of this malady. Insulin insufficiency and hyperglycaemia though are the primary launching pads, many other secondary consequences like metabolic derangement, oxidative stress, hyperlipidemia etc. contribute to many other manifestations affecting the cardiovascular system, kidney, retina, nerve, lens and skin. Overall, quality of life suffers and longevity gets curtailed (Moritum et al., 2003). Multiple foci of insulin sensitive function such as glycogenesis, glycogenolysis, gluconeogenesis, glucose uptake and transport are all impaired.

Apart from impaired glucoregulation and metabolic derangement, oxidative stress is another contributing factor in the many life-threatening complications linked with diabetes. Enough evidence is available to show that diabetic patients are under oxidative stress and that, increased oxidative stress contributes to the development and progression of diabetes and associated complications (Bonnefont-Rousselot et al., 2000; Maritum et al., 2003). Hyperglycaemia not only engenders free radicals but also impairs endogenous antioxidant defence system (Saxena et al., 1993; Maritum et al., 2003). Compromised antioxidant system denoted by increased lipid peroxidation and decreased levels of both non-enzymatic and enzymatic antioxidants is a feature of diabetes and, such changes find expression in alloxan or
Any therapeutic approach should therefore have the competence to have a holistic impact to counter or alleviate the many secondary complications besides, ameliorating the primary causes of diabetes. Exercise, along with dietary restriction and medication has been advocated as of great relevance for diabetic patients (Joslin et al., 1959). The American Diabetes Association (1997) has recommended regular but controlled exercise to diabetic patients as, it is purported to have beneficial effects reducing the risk of secondary complications. The intensity of recommended exercise may however vary from individual to individual depending on severity of diabetic complications and overall health of the individual. Exercise is now gaining greater acceptance as a therapeutic approach in various metabolic disorders like diabetes and obesity due to its low cost and non-pharmacological action (Normand et al., 2001). Accordingly, an exercise regimen of swimming for 30 minutes daily for 15 days has shown favourable influence in diabetic animals (Chapter -8).

Several studies have shown the protective effect of melatonin against streptozotocin induced pancreatic β cell damage and the subsequent development of Type I Diabetes (Montilla et al., 1998; Anderson and Sandler, 2001; Abosy et al., 2003; Anwar and Meki, 2003; Yavug et al., 2003; Gorgun et al., 2004). Further, diabetes associated increased generation of free radicals has been reported (Bayber, 1991). The contributing factors for the formation of these radicals are likely to be, increased non-enzymatic and auto-oxidative glycosylation, metabolic stress
due to changes in energy metabolism, levels of inflammatory mediators and, status of antioxidant defence (Griesmacher et al., 1995). The role of pineal and melatonin in glucoregulation and carbohydrate metabolism has received attention almost for the last five decades. Many of the earlier studies falling in the later half of the last century, have suggested the possible role for pineal and melatonin in pancreatic function, insulin secretion, glycaemic regulation, carbohydrate metabolism, glucose uptake and insulin sensitivity (Ramachandran, 2003; Peschke, 2008). Apart from its normal role in preventing oxidative stress, melatonin has now received recognition as a powerful antioxidant even under various pathological conditions due to its several mechanisms of action (see Tomas-Zapico and Coto-Montes, 2007). In this context, melatonin treatment was, tried out, to assess its therapeutic value in diabetic animals, which showed a dosage dependent differential effect (Chapter 2). Melatonin did show holistic effects in correcting various facets of diabetic complications.

In keeping with these findings, the present study attempts to assess a combination effect of a low dose of melatonin and swimming in amelioration of diabetic manifestations on a holistic basis.
Results

Glycaemic status (Table 9.3)

A combination of exercise and melatonin had no significant effect in the fed state of glycaemic status in NC animals but significant hypoglycaemia was the feature in the starved state. Diabetic induction was marked by significant hyperglycaemia which was significantly brought down by E+M in both fed and fasted states.

Glucose tolerance test (GTT) (Figs. 9.1, 9.2; Table 9.5)

Improved glucose tolerance in DC+S+M animals is indicted by the significantly lowered position of the tolerance curve compared to DC animals and the significantly decreased area under curve. The glucose clearance value of DC+S+M is the highest, denoting glucose induced insulin release.

Insulin response test (IRT) (Figs. 9.3, 9.4; Table 9.6)

A cursory glance at the insulin response curves shows a distinctly higher position of DC curve. The curves of NC and NC+S+M animals were indistinguishable and place low and DC+S+M curve can be seen merged with them. This is supported by the significantly reduced area under curve, identical with that of NC and NC+S+M animals. Insulin induced glucose clearance rate calculated for all groups of animals shows the highest value to be of DC+S+M, denoting greater insulin sensitivity.

Carbohydrate metabolism (Figs. 9.6, 9.7; Table 9.7)

Diabetic animals showed a significantly decreased hepatic and muscle glycogen contents while, a combination of S+M showed significant increase in tissue glycogen contents in both NC and DC animals. Corresponding to the glycogen contents, hepatic and muscle phosphorylase activity increased significantly in DC animals.
and, S+M decreased the enzyme activity in both NC and DC groups. Hepatic G-6-
pase activity was also significantly increased in DC animals and decreased in NC
and DC animals subjected to exercise and melatonin supplementation.

**Tissue Protein content (Table 9.7)**

Both NC+S+M and DC animals showed significant reduction in hepatic, muscle and
kidney protein contents, more significantly in the latter. Diabetic animals subjected to
exercise and given supplementation of melatonin significantly increased the tissue
protein contents to NC levels.

**Tissue cholesterol and lipid contents (Table 9.8)**

In general, hepatic, muscle and kidney lipid and cholesterol contents increased
significantly in diabetic animals. Whereas S+M decreased tissue lipid contents in
both NC and DC animals, tissue cholesterol contents were increased in NC animals
and decreased in DC animals.

**Serum lipid profile (Table 9.9)**

The serum levels of TG, TC, LDL and VLDL were increased significantly in diabetic
animals. Whereas TG was increased and cholesterol fractions decreased by S+M in
NC animals, both were significantly decreased in DC animals.

**Serum hormone profile (Table 9.10)**

**Corticosterone (Cort), Insulin, Oestrogen (E2) and Progesterone (P4)**

Serum insulin titre was significantly decreased in DC animals. However, insulin level
was increased in both NC+S+M and DC+S+M. In contrast, Cort was significantly
increased in Dc but decreased in NC+S+M and DC+S+M. Oestradiol was increased
in all experimental animals in the order DC+S+M>DC>NC+S+M. In contrast, P4 was 
decreased in all experimental groups in the order NC+S+M >DC>DC+S+M.

**Oxidative stress parameters**

**Lipid peroxidation (LPO) (Fig. 9.8)**
Lipid peroxidation levels of liver, muscle and kidney showed an identical pattern of 
significant increase in DC animals and reduction in both NC+S+M and DC+S+M.

**Non-enzymatic antioxidants (Table 9.11)**

**Reduce glutathione (GSH)**
Hepatic, muscle and kidney GDH contents were decreased significantly in DC 
animals. Whereas, S+M schedule decreased tissue GSH content in NC animals, it 
increased tissue GSH contents in DC animals.

**Enzymatic antioxidants (Table 9.12)**

**Catalase (cat), Superoxidase dismutase (SOD) and Glutathione peroxidase**
(GPx)
Diabetic animals showed significantly decreased activity levels of Cat, SOD and GPx 
in liver, muscle and kidney. Whereas Cat and SOD were decreased, GPx was 
increased in NC+S+M animals. The activities of GPx and Cat were increased in 
DC+S+M animals while, SOD activity was decreased in liver and muscle and 
increased in kidney.

**Serum markers of hepatic function (Table 9.13)**

**SGPT, SGOT, ALP and ACP.**
All the four serum markers were significantly increased in DC animals but were 
reduced in NC+S+M and DC+S+M, significantly more in the latter.
Serum markers of renal function (Figs. 9.9, 9.10)

Urea and Creatinine

In general, both the markers were significantly increased in diabetic animals and decreased in NC+S+M and DC+S+M animals.

Immunoblot analysis of GLUT-4 (Figs. 9.5A, B)

The noticeable feature was the significant decrement in cytosolic GLUT-4 in DC animals and the significant increase in DC+S+M animals.

Histology of Pancreas (Plate 9)

Diabetic pancreas was marked by histologically visible islet disruption with loss of islet cells and rampant intercellular spaces. A combination of exercise and melatonin supplementation improved the islet morphology to a more robust looking one in NC animals while, it rejuvenated the diabetic islet marked by more dense mass of islet cells.
### Table 9.1: Body weight (g), food (g) and water (ml) intake in all the experimental groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>INITIAL BW</th>
<th>FINAL BW</th>
<th>FOOD INTAKE</th>
<th>WATER INTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>253.3±5.9</td>
<td>263.3±7.27</td>
<td>16.15±0.01</td>
<td>35.56±1.15</td>
</tr>
<tr>
<td>NC+S+M</td>
<td>245.2±6.21</td>
<td>258.5±5.57</td>
<td>19.32±0.021</td>
<td>40.12±1.11</td>
</tr>
<tr>
<td>DC</td>
<td>186.67±17.09</td>
<td>189.86±6.012</td>
<td>25.13±0.33</td>
<td>73.5±0.12</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>240.44±14.20</td>
<td>245.56±6.77</td>
<td>20.12±0.21</td>
<td>67.11±0.87</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin

a) p<0.05, b) p<0.025, c) p<0.01, d) p<0.005, e) p<0.0005 compared to NC

### Table 9.2: Relative weights (g/100g of body weight) of liver, muscle, kidney, spleen and adrenal of all the experimental groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>LIVER</th>
<th>MUSCLE</th>
<th>KIDNEY</th>
<th>SPLEEN</th>
<th>ADRENAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>2.22±0.14</td>
<td>0.49±0.01</td>
<td>1.68±0.033</td>
<td>0.31±0.07</td>
<td>0.018±0.001</td>
</tr>
<tr>
<td>NC+S+M</td>
<td>2.56±0.012</td>
<td>0.51±0.001</td>
<td>1.65±0.022</td>
<td>0.30±0.005</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>DC</td>
<td>3.36±0.001c</td>
<td>1.015±0.02d</td>
<td>0.89±0.01e</td>
<td>0.27±0.006</td>
<td>0.031±0.001</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>3.2±0.002</td>
<td>0.98±0.0024</td>
<td>1.24±0.001@</td>
<td>0.29±0.001</td>
<td>0.025±0.002</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+E+M = Non Diabetic Control+Polyherbal Extract+Swimming+Melatonin
DC = Diabetic Control, DC+S+E+M = Diabetic Control+Swimming+Polyherbal Extract+Melatonin

a) p<0.05, b) p<0.025, c) p<0.01, d) p<0.005, e) p<0.0005 compared to NC
Table 9.3: Levels of Fasting and Fed Serum Glucose (mg/dl) in Exercised and Melatonin Treated Non Diabetic and Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>FASTING</th>
<th>FED</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>93.97±3.50</td>
<td>113.36±3.18</td>
</tr>
<tr>
<td>NC+M+S</td>
<td>74.66±2.143c</td>
<td>113.33±2.38</td>
</tr>
<tr>
<td>DC</td>
<td>443.33±31.21c</td>
<td>655.33±7.69c</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>100±2.880#</td>
<td>210±2.960g</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin

a)p<0.05, b)p<0.025,c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, °p<0.005 •) p< 0.0005 compared to DC

Table 9.4: Serum hormone profile of exercised and Melatonin treated non diabetic and diabetic rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>INSULIN pg/ml</th>
<th>CORTICOSTERONE ng/ml</th>
<th>ESTRADIOL pg/ml</th>
<th>PROGESTERONE ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.34±0.01</td>
<td>8.38±0.59</td>
<td>0.19±0.01</td>
<td>66.68±3.48</td>
</tr>
<tr>
<td>NC+M+S</td>
<td>0.44±0.02c</td>
<td>6.70±0.431b</td>
<td>0.32±0.001c</td>
<td>22.33±0.002</td>
</tr>
<tr>
<td>DC</td>
<td>0.16±0.01*</td>
<td>25.0±1.45*</td>
<td>1.98±0.0012*</td>
<td>54.68±1.74c</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>0.27±0.01®</td>
<td>18.13±0.002®</td>
<td>2.32±0.081®</td>
<td>59.18±0.08®</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin

a)p<0.05, b)p<0.025,c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, °p<0.005 •) p< 0.0005 compared to DC
Figure 2.1: Serum glucose levels in response to oral glucose tolerance test (OGTT) within a time range of 0 to 120 minutes in all the experimental groups.

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC= Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin

\( a)p<0.05, \ b)p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 \) compared to NC and \( *p<0.05, \ #p<0.025, @) p< 0.01, \ &p<0.005 \) compared to DC

Figure 9.2: Area under curve for OGTT in all experimental groups
Figure 9.3: Serum glucose levels in response to insulin administration (IRT) within a time range of 0 to 120 minutes of all the experimental groups.

Data are expressed as Mean±SE

NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC= Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin

a)p<0.05, b)p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, p<0.005 •) p< 0.0005 compared to DC

Figure 9.4: Area under curve for IRT in all experimental groups.

NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC= Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin
Table 9.5: Elevation and clearance rates of glucose during OGTT in Exercised and Melatonin diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>RATE OF ELEVATION</th>
<th>RATE OF CLEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.21</td>
<td>0.50</td>
</tr>
<tr>
<td>NC+S+M</td>
<td>1.05</td>
<td>0.04</td>
</tr>
<tr>
<td>DC</td>
<td>9.13</td>
<td>1.97</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>6.6</td>
<td>4.41</td>
</tr>
</tbody>
</table>

Table 9.6: Clearance and elevation rates of glucose during IRT in Exercised and Melatonin treated diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>RATE OF CLEARANCE</th>
<th>RATE OF ELEVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>2.1</td>
<td>0.56</td>
</tr>
<tr>
<td>NC+S+M</td>
<td>1.49</td>
<td>0.279</td>
</tr>
<tr>
<td>DC</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>DC+CD+M</td>
<td>2.65</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 9.7: Tissue protein and glycogen content (mg/100 mg tissue) in Control and Treated Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>PROTEIN</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td>NC</td>
<td>16.39±1.24</td>
<td>9.76±1.41</td>
</tr>
<tr>
<td>NC+M + S</td>
<td>15.77±0.84</td>
<td>8.47±0.17</td>
</tr>
<tr>
<td>DC</td>
<td>13.90±0.88</td>
<td>5.50±0.73c</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>16.48±1.22</td>
<td>6.36±0.350</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin, DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin

a) p<0.05, b) p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, °p<0.005 •) p< 0.0005 compared to DC
GLUCOSE 6 PHOSPHATASE

EXPERIMENTAL GROUPS

GLYCOGEN PHOSPHORYLASE

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin
a)p<0.05, b)p<0.025, c)p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, p<0.005 •) p< 0.0005 compared to DC

Figure 9.5: Hepatic and muscle glycogen phosphorylase activity in exercised and Melatonin treated non diabetic and diabetic rats

Figure 9.6: Hepatic Glucose-6-phosphatase activity in exercised and Melatonin treated non diabetic and diabetic rats
Table 9.9: Tissue cholesterol and lipid content (mg/100mg tissue) in Control and Treated Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Liver</th>
<th>Muscle</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.28±0.005</td>
<td>0.124±0.010</td>
<td>0.37±0.030</td>
<td>4.21±0.72</td>
<td>1.56±0.43</td>
<td>0.733±0.06</td>
</tr>
<tr>
<td>NC+M+S</td>
<td>0.36±0.072</td>
<td>0.14±0.011</td>
<td>0.21±0.025 c</td>
<td>3.51±0.61 c</td>
<td>1.01±0.31</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>DC</td>
<td>0.6±0.004 e</td>
<td>0.29±0.03 e</td>
<td>0.58±0.05 e</td>
<td>6.32±0.02 e</td>
<td>2.08±0.41</td>
<td>0.93±0.04 e</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>0.45±0.020 e</td>
<td>0.29±0.023</td>
<td>0.52±0.027 e</td>
<td>5.17±0.71 e</td>
<td>1.73±0.23 e</td>
<td>0.81±0.06</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin
a)p<0.05, b)p<0.025, c)p<0.01, d)p<0.005, e)p<0.0005 compared to NC
*p<0.05, #p<0.025, @)p<0.01, *p<0.005 compared to DC
Table 9.8: Serum lipid profile (mg/dl) of exercised and Melatonin treated non diabetic and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>80±2.31</td>
<td>68.67±3.44</td>
<td>50.66±1.76</td>
<td>15±1.73</td>
<td>13.11±1.73</td>
</tr>
<tr>
<td>NC+M+S</td>
<td>78±2.64</td>
<td>94.02±1.05^c</td>
<td>40.77±2.31^c</td>
<td>20.5±1.58^b</td>
<td>16.53±0.40^a</td>
</tr>
<tr>
<td>DC</td>
<td>97.33±4.34^d</td>
<td>140.67±2.3^a</td>
<td>45.4±2.6^c</td>
<td>30.6±0.87^e</td>
<td>22.2±2.90^b</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>91.66±2.73@</td>
<td>92.22±1.15@</td>
<td>49.15±0.50</td>
<td>27.5±1.30@</td>
<td>15.66±0.65@</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin
a)p<0.05, b)p<0.025, c)p<0.01, d)p<0.005, e)p<0.0005 compared to NC and *p<0.05, #p<0.025, @)p<0.01, p<0.005 •)p<0.0005 compared to DC

Figure 9.7: Levels of LPO in Exercised and Melatonin treated diabetic and non diabetic rats

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin
DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin
a)p<0.05, b)p<0.025, c)p<0.01, d)p<0.005, e)p<0.0005 compared to NC and *p<0.05, #p<0.025, @)p<0.01, •)p<0.0005 compared to DC
Table 9.10 Enzymatic anti-oxidant status of exercised and Melatonin treated non-diabetic and diabetic treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPX</th>
<th>CAT</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
<td>Kidney</td>
</tr>
<tr>
<td>NC</td>
<td>4.59±0.83</td>
<td>12.45±1.60</td>
<td>2.16±0.18</td>
</tr>
<tr>
<td>NC+M+S</td>
<td>8.36±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.34±1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.73±0.48&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC</td>
<td>4.64±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.56±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>5.55±0.30&lt;sup&gt;@&lt;/sup&gt;</td>
<td>17.66±0.99&lt;sup&gt;@&lt;/sup&gt;</td>
<td>7.53±0.40&lt;sup&gt;@&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE.
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin, DC = Diabetic Control, DC+S+M = Diabetic Control+Swimming+Melatonin.

*<sup>a</sup>/p<0.05, b/p<0.025, c/p<0.01, d/p<0.005, e/p<0.0005 compared to NC and *<sup>p</sup>/p<0.05, #<sup>p</sup>/p<0.025, @/p<0.01, **/p<0.005 compared to DC.
Table 9.11: Tissue non-enzymatic anti-oxidant status (mg/100 mg tissue) in exercised and Melatonin treated non diabetic and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Muscle</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>31.14±2.58</td>
<td>14.58±1.51</td>
<td>25.03±1.15</td>
</tr>
<tr>
<td>NC+M + S</td>
<td>27.77±1.49</td>
<td>13.56±0.83</td>
<td>26.71±0.67</td>
</tr>
<tr>
<td>DC</td>
<td>11.01±1.29*</td>
<td>13.05±1.38</td>
<td>13.04±1.86*</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>15.24±1.54*</td>
<td>21.64±0.85®</td>
<td>21.12±1.52®</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+Swimming+ Melatonin
a) p<0.05, b) p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, †p<0.005
•) p< 0.0005 compared to DC

Table 9.12: Serum Markers of Hepatic Dysfunction in Control and Extract Treated Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT U/L</th>
<th>SGOT U/L</th>
<th>ALP U/L</th>
<th>ACP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>40±4.04</td>
<td>71.33±2.96</td>
<td>204±2.64</td>
<td>8.5±0.86</td>
</tr>
<tr>
<td>NC+M + S</td>
<td>37.64±3.22</td>
<td>68.47±3.06</td>
<td>109.41±4.41</td>
<td>8.94±0.44</td>
</tr>
<tr>
<td>DC</td>
<td>125±5.87*</td>
<td>290.67±5.79*</td>
<td>471.67±2.34</td>
<td>12.20±0.61†</td>
</tr>
<tr>
<td>DC+S+M</td>
<td>35.28±1.49®</td>
<td>69.56±1.36®</td>
<td>150.29±1.84®</td>
<td>11.26±0.68</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+Swimming+ Melatonin
a) p<0.05, b) p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, †p<0.005
•) p< 0.0005 compared to DC
Figure 9.8: Serum Urea level in Exercised and Melatonin treated non diabetic and diabetic rats

![Urea graph]

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC = Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin
a)p<0.05, b)p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, •) p< 0.0005 compared to DC

Figure 9.9: Serum Creatinine level in all the experimental groups.

![Creatinine graph]

Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC = Diabetic Control, DC+S+M = Diabetic Control+ Swimming+ Melatonin
a)p<0.05, b)p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p< 0.01, •) p< 0.0005 compared to DC
Data are expressed as Mean±SE
NC = Non Diabetic Control, NC+S+M = Non Diabetic Control+Swimming+Melatonin DC= Diabetic Control, DC+S+M = Diabetic Control+Swimming+ Melatonin
a)p<0.05, b)p<0.025, c) p<0.01, d) p< 0.005, e) p< 0.0005 compared to NC and *p<0.05, #p<0.025, @) p<0.01, p<0.005
* p< 0.0005 compared to DC

**Fig. 9.10 (A)** Immunoblot analysis of Glut-4 protein expression. (B) Semi-quantification analysis of Glut-4 protein using scanning densitometry. Signals of Glut-4 in immunoblot were quantified arbitrarily. Bars represent means of ± S.E. of independent experiments and a representative immunoblot is shown here.
PLATE 9

Figure A: Transverse section of pancreas of non diabetic rat showing an islet. Note the intact islet histoarchitecture (450X)

Figure B: Transverse section of pancreas of rat subjected to exercise along with melatonin supplementation. Note the better looking islets (450X)

Figure C: Transverse section of pancreas of diabetic rat. Note islet cell destruction and the wide intercellular spaces. (450X)

Figure D: Transverse section of pancreas of diabetic rat subjected to exercise along with melatonin supplementation. Note the islet architecture with dense population of islet cells suggesting significant protection against alloxan insult. (450X)
Discussion:
The present holistic evaluation on the effect of a combination of melatonin (M) and swimming exercise (S), on various facets of diabetic manifestations, has shown an overall beneficial effect with some effects resembling those of exercise, others of melatonin and yet others of a cumulative additive effect of both melatonin and exercise. The non-diabetic animals have shown a significantly greater hypoglycaemic effect in the fasted state and normoglycaemia in the fed state. The average glycaemic status of fed and fasted taken together is lowest compared to exercise or melatonin alone. Remarkably, the effect of M+S in lowering glycaemic status of starved non-diabetic animals is, significantly greater in comparison to M or S alone. The hepatic and muscle glycogen contents have also shown significant increase. The increase in muscle glycogen content is to the same degree in all the three schedules while, the increase in hepatic glycogen content is more with melatonin than with exercise and, the combination of exercise and melatonin has no additive effect over that of melatonin. The related changes in G-6pase and phosphorylase are in agreement with the changes in glycogen contents and, their decreased activities are similar to those recorded for melatonin. The present observations on carbohydrate metabolism tend to suggest a favourable influence of M+S in increasing tissue load of glycogen that is more relatable with the effect of melatonin. The recorded increase in insulin level is in agreement with the observed changes in carbohydrate metabolism and akin to that of exercise alone. Though the increased insulin levels is well reflected in the higher percentage of glycogen deposition in the liver compared to E or M alone, it is not clear as to whether the increase in insulin titre is due to an increased pancreatic release or due to
decreased metabolic clearance of the hormone from the blood stream. Apparently, E+M in non-diabetic animals has no significant effect in relation to glycaemic regulation and glycogen metabolism. However, glucose uptake and glycogenesis seem to be augmented as seen by the decrease in cytosolic GLUT-4 levels and increase in tissue glycogen loads. The decrease in cytosolic GLUT-4 is suggestive of increased membrane translocation. Increased GLUT4 expression and glucose uptake under exercise regimen have been reported (Han and Pronen, 1998; Richter et al., 2001).

The presently observed increase in serum triglyceride coupled with significant depletion of tissue lipids is suggestive of increased lipid mobilization in the wake of exercise-induced increase in energy demand. Though the decrease in tissue lipids is indication of increased lipolysis, the increase in serum TG levels attests to under utilization. Apparently, the balance seems tilted more towards lipolysis rather than lipid utilization. It is presumable that, continuous regular exercise marked by gradual shift towards lipid utilization with persistent chronic exercise (duration dependent and the type and degree of exercise activity), gradual adaptation sets in wherein, efficient lipid utilization ultimately keeps pace with lipid mobilization. With exercise induced metabolic adaptations, lowered serum lipids could be the feature as shown by other workers studying exercise adaptation (Horowiz and Klien, 2000; Aginilo et al., 2003; Hernandez-Torres et al., 2009). It is also likely that, sub maximal exercise insufficient to bring about adaptive change, could result in higher levels of plasma glucagon and Catecholamines, which could contribute to increased lipolysis and higher serum lipid levels. In fact, Winder et al. (1979) have recorded increased plasma glucagon and catecholamines in individuals subjected to sub maximal exercise.
Now the presently observed changes in serum triglycerides and tissue lipids, is more of a potentiated S effect in presence of M (Chapter 8). In contrast, the increased tissue cholesterol contents and decreased serum cholesterol levels are more of M effect slightly underplayed by S (Chapter 2).

Previously (Chapter 8), it was shown that, the present exercise regimen does generate certain degree of oxidative stress in non-diabetic animals as marked by a depletion in GSH content and decreased activities of Catalase and SOD. In the present study as well, this S induced effect is visible, though to a lesser extent. Apparently, simultaneous melatonin treatment protects against exercise-induced decrease in GSH content and Catalase and SOD activities. Inconclusive results are seen in literature regarding exercise induced oxidative stress as, some suggest induction of oxidative stress and, others show an up-regulation of antioxidant status (Sen, 1995; Urso and Clarkson, 2003; Gomez-Cabrera et al., 2008). Apparently, initial phases of sub maximal training exercise prior to adaptation may result in some oxidative stress while, long-term exercise may lead to adaptation and favourable whole body antioxidant status. However, melatonin seems capable of minimizing the initial oxidative stress caused due to sub maximal exercise prior to setting in of adaptation. This favourable influence of melatonin is in keeping with its recognized role as an antioxidant (See Tomas-Zapico and Coto-Montas, 2007).

Previous study has shown an increase in serum markers of renal and hepatic dysfunction under the present exercise regimen (Chapter 8). However, the administration of melatonin in the present study in conjunction with the same exercise paradigm, has shown a favorable effect and minimized or even neutralized the elevation in serum markers of urea, creatinine, SGPT, SGOT,
ALP and ACP induced by swimming exercise, which is in keeping with the known cytoprotective effect of melatonin (Das et al., 2008).

The experimental paradigm of S+M appears to be significantly more beneficial to diabetic animals as can be realized from the recorded observations. Melatonin treated exercised animals subjected to an overnight fast has significant favourable influence as marked by the normoglycaemic status in such animals, seemingly a swimming exercise-induced effect (Chapter 8). The effect of exercise in melatonin treated fed diabetic animals, is also very significant as, the glycaemic status was only 122% higher compared to the 595% higher glycaemic status in diabetic animals. Since the diabetic animals subjected to exercise alone still showed 539% higher glcaemic level as against only 190% higher glycaemic level in melatonin treated diabetic animals, the presently observed 122% higher glycaemic status in S+M diabetic animals suggests a potentiation of M effect with concurrent exercise regimen. The significant hypoglycaemic effect of S+M in diabetic animals is well co-relatable with the recorded significant glucose elevation rate under GTT, as good as diabetic S animals, and a much better clearance rate followed by elevation rate than both S and M diabetic animals, is indicative of the highly favorable influence of exercise in conjunction with melatonin treatment for amelioration of diabetic hyperglycaemia. Overall, the glucoregulatory effects of S+M is more of an S effect potentiated further by M and, some recent studies do suggest favourable influence of exercise on glucoregulation and hormonal changes (Guelfi et al., 2005; Harmer et al., 2007 Gulve, 2008). The observed better glucose elevation rate subsequent to an insulin challenge induced glucose clearance in the diabetic animals, attests to potentiated insulin sensitivity and glucose disposal from circulation, as well as of,
improved glucagon response to an insulin challenge that is compromised in diabetic animals (Quesada et al., 2008).

The favorable influence of S+M in glucose clearance of diabetic animals seen in the present study, finds positive co-relations with the recorded increment in tissue glycogen load and decrease in Glucose-6-Phosphatase and Glycogen Phosphorylase activities. These changes in carbohydrate metabolism are truly more of an exercise effect rather than of Melatonin (Chapters 2 & 8). The purported significantly augmented glucose disposal in diabetic rats treated with M and, subjected to S is well supported by the greatly improved serum insulin status, which is much better than that seen with S or M alone. The favorable influence on glucoregulation seems to be due to both an improved insulin titre as well insulin sensitivity. The very significant glucoregulatory effect despite the still persisting insulin deficit, bespeaks of the possible insulin independent mechanism of glucose uptake, a mechanism essentially attributable to exercise. This is well substantiated by the significant increase in GLUT-4 levels induced by a combination of S and M from the diabetic low. Apparently, increased GLUT-4 gene expression as well as membrane translocation are inferable, as has been demonstrated by the many studies showing exercise induced increased GLUT-4 translocation as well as GLUT-4 mRNA expression in both diabetic and non-diabetic animals or humans (Chabalin et al., 2000; Kraniou et al., 2000, 2006; MacLean et al., 2000, 2002; Tomas et al., 2002; Barth et al., 2004). The increased glucose uptake seen in the present study despite the insufficiency of insulin, suggests the participation of alternate signalling molecules that could activate GLUT-4 vesicle translocation. In this context, many recent studies have provided evidence for AMPK and cAMP K mediated excercise induced insulin
independent signalling pathway in GLUT-4 translocation to membrane (Hayashi et al., 1997; Musil et al., 2001; Richter et al., 2001; Tomas et al., 2002; Holloszy, 2003; Jose and Richter, 2005).

The favourable influence of a combination, of exercise and melatonin seen with reference to diabetic glycaemic status, glucose uptake and carbohydrate metabolism, is also well reflected in the improved lipid profile. Both, tissue cholesterol and lipids, as well as serum cholesterol, LDL, VLDL, and TG have all shown significant reduction in melatonin treated exercised diabetic rats suggesting a lipid lowering effect of a combination of exercise and melatonin in diabetic animals. Unlike in non diabetic animals, a 15 day schedule of swimming exercise along with melatonin administration appears to be adaptive in diabetic animals marked by carbohydrate conservation and lipid utilization. It is likely that, blood glucose may also serve as an adequate metabolic substrate along with lipids. Unlike in non diabetic animals where the 15 day exercise schedule was found to be non adaptive marked by increased lipid mobilization rather than utilization, the same schedule in diabetic animals is adaptive and tilts the scale towards greater lipid utilization than mobilization. Supportive evidence comes from many studies demonstrating lowered lipid profile in exercised diabetic subjects (Laakosonen et al., 2000, 2003; Gordon et al., 2008). The lipid and cholesterol lowering effect of S+M is significantly greater than that of S or M alone, indicating a cumulative or additive effect of S and M.

Diabetic complications are marked by increased oxidative stress as denoted by increased LPO and decrease in content an activity of non-enzymatic and enzymatic antioxidant respectively. The present finding of significant decrement in LPO and increase in endogenous antioxidants with GPx depicting
even an above normal level, are in agreement with the many reports on exercise induced amelioration of oxidative stress (Gul et al., 2002; Maritim et al., 2003; Coskun et al., 2004; Black et al., 2005; Chang et al., 2007; Gordon et al., 2008). Interestingly, the effect of S+M on oxidative stress is more akin to that of S alone and, much less than that of M alone, suggesting a better antioxidant effect of melatonin in non exercised diabetic animals rather than in exercised diabetic animals.

Diabetes is marked by significantly increased levels of serum markers of hepatic and renal function. Previously it was shown that, swimming alone has a favourable influence on markers of hepatic function while, melatonin had favourable influence on both hepatic and renal markers (Chapters 8 and 2 respectively). A combination of S and M used in the present study is significantly more hepato and xeno protective as marked by significant reduction in the levels of serum markers of hepatic and renal functions, much more than that recorded for M or S alone. Increased serum oestrogen titre and reduced corticosterone levels in S+M diabetic animals compared to diabetic animals, is a favourable hormonal milieu in support of amelioration of oxidative stress and cytoprotection. In conclusion, the present observations tend to suggest an overall favourable cumulative, additive influence of S and M in gluoregulation, lipid profile and hepatic and renal functions in animals with Type I diabetes. Based on these, a combination of exercise and melatonin may be of therapeutic value in ameliorating diabetic manifestations.