5.1. Hypoglycemic effect of chrysin on STZ-NA induced experimental T2DM in rats

5.1.1. Dose determination study for chrysin in diabetic rats

Figure 6 illustrates the hypoglycemic effect of chrysin at different concentrations. Diabetic control rats showed significantly (p<0.001) increased levels of blood glucose which reduced significantly (p<0.001) on oral administration of chrysin for 28 days at different doses. Chrysin at a dose of 50mg/kg and 100mg/kg exhibited maximum blood glucose lowering effect than at 25mg/kg. Hence, further the optimum dose of chrysin was fixed as 50mg/kg and the same dose followed for further study or followed in experimental groups.

Figure 6: Dose determination study for chrysin (Blood glucose)

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group II. ***p< 0.001.
5.1.2. Effect of chrysin on food and water intake in experimental rats

Increased intake of food and water was observed in STZ-NA (Group III) induced rats. This was inhibited significantly (p<0.001) and maintained to normal on chrysin treated diabetic (Group IV) rats which was depicted in figure 7 (a) and (b).

Figure 7: Effect of chrysin on food and water intake in experimental rats

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.1.3. Effect of chrysin on body weight

Table 4 represents changes in the body weight of normal and diabetic rats at regular intervals (1\textsuperscript{st}, 7\textsuperscript{th}, 14\textsuperscript{th}, 21\textsuperscript{st}, 28\textsuperscript{th} days). During the experimental period, body weight significantly (p<0.001) decreased in diabetic rats compared to the normal control rats. Oral administration of chrysin in diabetic induced rats prevented the loss of body weight and the results were compared with standard antidiabetic drug glibenclamide rats.

Table 4: Effect of chrysin on body weight (gms) in experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>158.33±6.06</td>
<td>160.00±6.23</td>
<td>165.83±4.54</td>
<td>169.33±5.92</td>
<td>175.83±8.64</td>
</tr>
<tr>
<td>Group II</td>
<td>155±4.47\textsuperscript{ms}</td>
<td>157.17±2.64\textsuperscript{ms}</td>
<td>161.83±4.22\textsuperscript{ms}</td>
<td>167.33±3.72\textsuperscript{ms}</td>
<td>172.33±4.68\textsuperscript{ms}</td>
</tr>
<tr>
<td>Group III</td>
<td>150±3.16\textsuperscript{***}</td>
<td>147.16±2.56\textsuperscript{***}</td>
<td>145.33±5.89\textsuperscript{***}</td>
<td>144.00±7.75\textsuperscript{***}</td>
<td>138.50±1.63\textsuperscript{***}</td>
</tr>
<tr>
<td>Group IV</td>
<td>151.67±5.16\textsuperscript{b**}</td>
<td>151.17±4.45\textsuperscript{b**}</td>
<td>154.67±5.54\textsuperscript{b**}</td>
<td>159.67±5.24\textsuperscript{b**}</td>
<td>165.17±5.19\textsuperscript{b**}</td>
</tr>
<tr>
<td>Group V</td>
<td>155.00±3.16\textsuperscript{b**}</td>
<td>157.17±2.04\textsuperscript{b**}</td>
<td>160.83±3.25\textsuperscript{b**}</td>
<td>164.00±2.37\textsuperscript{b**}</td>
<td>168.83±2.32\textsuperscript{b**}</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III). ***p<0.001; **p<0.01; ns – non significant.

5.1.4. Effect of chrysin on oral glucose tolerance test in experimental rats

Figure 8 illustrates the effect of chrysin on OGTT at different time points in 2 hours. In diabetic rats, the blood glucose reached peak at 1 hour after the oral glucose administration, and remained higher even after 2 hours despite slight decreased. On chrysin treatment, the glucose level was maintained at all time points similar to normal rats. Chrysin alone treated rats did not show significant increase in the levels of glucose for 2 hours.
Figure 8: Effect of chrysin on OGTT in experimental rats after 28 days

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. **p<0.001; *p<0.01; ns – non significant.

5.1.5. Effect of chrysin on blood glucose, plasma insulin and urine sugar

Table 5 illustrates the effect of chrysin on fasting blood glucose and plasma insulin levels in control and experimental groups. Diabetic rats showed significant (p<0.001) increase in blood glucose and (p<0.001) decrease in plasma insulin levels as compared with control rats. Diabetic rats on chrysin and glibenclamide treatment, showed significant decrease in blood glucose and increase in plasma insulin levels. Also increase in urine sugar level in diabetic induced rats was decreased on chrysin treatment.
Results

Table 5: Effect of chrysin on the levels of blood glucose and plasma insulin in experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood Glucose (fasting)</th>
<th>Plasma Insulin (μU/ml)</th>
<th>Urine Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day (mg/dl)</td>
<td>28th day (mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>73.83±6.11</td>
<td>77.00±3.35</td>
<td>12.65±0.48</td>
</tr>
<tr>
<td>Group II</td>
<td>72.00±6.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.00±5.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.09±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>366.5±12.61&lt;sup&gt;***&lt;/sup&gt;</td>
<td>359.33±14.62&lt;sup&gt;***&lt;/sup&gt;</td>
<td>7.99±0.67&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>358.17±13.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.83±8.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.67±1.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>365.83±10.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>116.33±7.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.04±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. <sup>***</sup>p<0.001, ns – non significant.

5.1.6. Effect of chrysin on hemoglobin and glycosylated hemoglobin

Figure 9 (a) and (b) represent the effect of chrysin on haemoglobin and glycosylated haemoglobin content in control and experimental rats. Decrease in haemoglobin and increase in glycosylated haemoglobin was noted in STZ-NA induced rats. Treatment with chrysin to diabetic rats showed significant (p<0.01) increase in the level of haemoglobin and remarkable decrease (p<0.001) in the level of glycosylated haemoglobin which was compared to glibenclamide treated rats.
Figure 9: Effect of chrysin on hemoglobin and glycosylated hemoglobin in experimental rats after 28 days

(a) Hemoglobin

(b) Glycosylated Hemoglobin

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p<0.001; **p<0.01; ns – non significant.
5.1.7. Histopathological examination of pancreas, liver and kidney tissues in experimental rats

**Pancreas**

Histopathological examination on hematoxylin-eosin staining of the pancreatic tissues revealed the architecture of β-cells in experimental group as represented in figure 10 (a-e). Control rats 10 (a) and chrysin alone 10 (b) treated rats showed normal structure of pancreatic islets with cluster of β-cells. Diabetes induced 10 (c) rats showed small shrunken islets, degranulation, vacuolation in pancreatic islets with reduction of β-cell mass. Moreover, in diabetic rats treated with chrysin 10 (d) showed nearly normal architecture similar to glibenclamide treated rats 10 (e) due to the regeneration of β-cells.

**Liver**

Figure 11 (a-e) represents the histoarchitecture of hematoxylin-eosin staining of hepatic tissues of control and experimental rats. Normal control rats 11 (a) exhibiting liver parenchyma showed cords of hepatocytes around the central vein. Chrysin alone treated rats 11 (b) showed hepatocytes with sinusoidal cords around the central vein and portal tracts. Figure 11 (c) exhibits lesions, congestions and dilation with mild inflammatory cell infiltrate and distortion of hepatocytes around the central vein. Whereas, chrysin treated diabetic rats samples figure 11 (d) showed improvement in the hepatocytes similar to hepatic architecture figure (e).
Figure 10: Photomicrographs of hematoxylin–eosin staining of pancreatic tissues in control and experimental rats. ×200 magnification. (a) Normal control (Group I) (b) Chrysin alone treated (Group II) (c) Diabetic control (Group III) (d) Diabetic chrysin-treated (Group IV) (e) Diabetic glibenclamide-treated (Group V)
Figure 11: Photomicrographs of hematoxylin–eosin staining of hepatic tissues in control and experimental rats. ×200 magnification. (a) Normal control (Group I) (b) Chrysin alone treated (Group II) (c) Diabetic control (Group III) (d) Diabetic chrysin-treated (Group IV) (e) Diabetic glibenclamide-treated (Group V)
Results

Figure 12: Photomicrographs of hematoxylin–eosin staining of renal tissues in control and experimental rats. ×200 magnification. (a) Normal control (Group I) (b) Chrysin alone treated (Group II) (c) Diabetic control (Group III) (d) Diabetic chrysin-treated (Group IV) (e) Diabetic glibenclamide-treated (Group V)
Results

Kidney

Figure 12 (a-e) represents the histoarchitecture of renal tissues of control and experimental rats. Control rats 12 (a) showed normal architecture of renal parenchyma with glomeruli and tubules surrounded by the Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes. 12 (b) Group II: animals also showed normal renal parenchyma with glomeruli and tubules with normal architecture. Renal parenchyma showing congestion blood vessels with inflammatory cell infiltrate, brush border loss and medullary congestions and interstitial tissue were noted in diabetic rats was shown in the figure 12 (c). Interestingly the chrysin treated rats shows renal parenchyma reduce the infiltrate in the interstitial tissue and reduced congestion in figure 12 (d) and from glibenclamide treated diabetic sample, study shows normal histology of renal tissue being comparable to normal control samples was shown the figure 12 (e).

5.2. The effect of chrysin on hyperglycemia mediated oxidative stress in STZ-NA induced experimental T2DM rats

5.2.1. Effect of chrysin on the activities of serum hepatic biomarkers in experimental rats

The effects of chrysin on serum hepatic marker enzymes are presented in table 6. The activities of serum marker enzymes AST, ALT and ALP were markedly elevated (p<0.001) in the diabetic rats. Conversely, oral administration of chrysin and glibenclamide treated rats significantly decreased (p<0.001) resulted in the restoration to normal level was comparable to that of control group. Control rats treated with chrysin alone did not showed any statistical significant difference on the levels comparison with normal control rats.
Results

Table 6: Effect of chrysin on serum hepatic biomarker enzymes in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>36.45±5.84</td>
<td>30.33±5.12</td>
<td>62.72±6.82***</td>
<td>42.57±7.50b***</td>
<td>36.95±2.38b***</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>32.58±5.26</td>
<td>31.78±4.35ns</td>
<td>68.21±6.34***</td>
<td>43.28±7.15b***</td>
<td>40.69±8.66b***</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>127.5±12.27</td>
<td>135.1±11.77</td>
<td>343.7±22.58a***</td>
<td>148.63±2.30b***</td>
<td>145.3±15.72b***</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. *** p<0.001; ns – non significant.

5.2.2. Effect of chrysin on the activities of serum renal biomarkers

The figures 13 (a), (b) and (c) summarize the activities of serum urea, serum uric acid and serum creatinine in normal, diabetic and chrysin treated groups. The activities of these molecules in serum were found to be significantly (p<0.001) increased when compared with normal control rats. Oral administration of chrysin treated diabetic (Group IV) rats and glibenclamide treated (Group V) rats recorded a significant (p<0.001) reduction in urea and uric acid and significantly (p<0.01) reduced creatinine to near normal level. No significant differences were observed in chrysin alone treated rats.
Results

Figure 13: Effect of chrysin on renal biomarkers in experimental rats

(a) Serum urea
(b) Serum uric acid
(c) Serum creatinine

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. *** p<0.001; **p<0.01; *p<0.05; ns – non significant.
5.2.3. Effect of chrysin on the activities of serum lipid profile in experimental rats

The levels of TC, TGs, HDLC, LDLC, and VLDLC for normal, diabetic and chrysin treated groups are presented in the table 7. Diabetic rats showed significant (p<0.001) increase in serum TC, TGs, LDLC, VLDLC level and significant (p< 0.001) decrease in serum HDLC level when compared to normal control rats. Administration of chrysin to diabetic rats and glibenclamide treated rats resulted a significant (p<0.001) restoration of the serum lipid content to near normal level. Normal chrysin treated rats did not exhibit significant differences of serum lipid profile parameters when compared with normal control rats.

Table 7: Effect of chrysin on serum lipid profile in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>42.03±5.28</td>
<td>39.40±4.95</td>
<td>65.43±7.68***</td>
<td>48.38±5.18b***</td>
<td>44.07±5.43b***</td>
</tr>
<tr>
<td>TGs (mg/dl)</td>
<td>40.55±4.20</td>
<td>39.52±5.46ans</td>
<td>80.93±5.40a***</td>
<td>41.77±5.10b***</td>
<td>36.5±3.51b***</td>
</tr>
<tr>
<td>HDLC (mg/dl)</td>
<td>40.57±3.19</td>
<td>40.33±3.89ans</td>
<td>26.34±5.54a***</td>
<td>36.9±2.66b***</td>
<td>41.33±2.98b***</td>
</tr>
<tr>
<td>LDLC (mg/dl)</td>
<td>24.35±3.50</td>
<td>24.82±2.99ans</td>
<td>51.72±3.28a***</td>
<td>29.64±4.14b***</td>
<td>28.46±4.17b***</td>
</tr>
<tr>
<td>VLDLC (mg/dl)</td>
<td>12.63±1.50</td>
<td>11.57±1.42ans</td>
<td>25.86±2.50a***</td>
<td>15.04±5.31b***</td>
<td>15.99±3.76b***</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p<0.001; ns – non significant.
Results

5.2.4. Effect of chrysin on enzymatic antioxidants in pancreatic tissue

Table 8 illustrates the effect of chrysin on the levels of enzymatic antioxidants SOD, CAT, GST and GPx in the pancreatic tissues of control and experimental groups of rats. The enzymatic antioxidant levels in pancreatic tissues of diabetic control rats were found to be significantly (p<0.001) reduced as compared with the normal control rats. Diabetic rats treated with chrysin for 28 days showed significant (p<0.001) increase in the levels of enzymatic antioxidants SOD, CAT, GST and GPx and restores to normal group I levels. However, chrysin administration to control (Group II) rats did not show any statistical difference than that of normal control (Group 1) rats.

Table 8: Effect of chrysin on enzymatic antioxidants in pancreatic tissue of experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>8.58 ± 3.34</td>
<td>8.77± 3.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.55±4.11&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>7.12± 2.82&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>7.43±10.45&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>45.65± 3.65</td>
<td>44.67±2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.43±1.54&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>43.53±3.76&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>42.53±1.44&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>GST</td>
<td>4.55 ± 2.10</td>
<td>4.22± 1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.15±1.04&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>3.54±0.94&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>3.04±0.47&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx</td>
<td>5.34 ± 1.32</td>
<td>5.70± 1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.53±2.54&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>4.79±1.02&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>4.76±1.22&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. ***p<0.001; ns – non significant

SOD: Units are expressed as: 50% inhibition of auto-oxidation of pyrogallol/min/mg protein; CAT: µmol of H₂O₂ consumed/ min/mg protein; GST: µmol of CDNB conjugated min/ mg protein; GPx: µmol of reduced GSH oxidized/min/mg protein.
5.2.5. Effect of chrysin on non-enzymatic antioxidants and lipid peroxidation in pancreatic tissue

The levels of non-enzymatic antioxidants GSH & vitamin C and LPO in pancreatic tissue were showed in the table 9. There was a significant (p<0.001) decrease in the GSH content & vitamin C and the level of MDA increased (p<0.001) significantly in diabetic rats when compared with normal control rats. MDA is a product of LPO and serves as an oxidative damage index. The GSH content and vitamin C in pancreatic tissue of chrysin treated diabetic rats increased significantly (p<0.001) and MDA decreased significantly (p<0.001) and those of glibenclamide treated rats when compared with diabetic control rats. There was no significant deviation found on the levels of GSH & vitamin C and LPO in rats treated with chrysin alone, when compared to group I rats.

Table 9: Effect of chrysin on non-enzymatic antioxidants and LPO in pancreatic tissue of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH</th>
<th>Vitamin C</th>
<th>LPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11.32 ± 1.34</td>
<td>2.01±0.13</td>
<td>6.54±2.54</td>
</tr>
<tr>
<td>Group II</td>
<td>12.65 ± 1.37\textsuperscript{a}</td>
<td>2.14±0.14\textsuperscript{a}</td>
<td>6.77±2.87\textsuperscript{a}</td>
</tr>
<tr>
<td>Group III</td>
<td>7.46 ± 2.54\textsuperscript{a,***}</td>
<td>1.21±0.21\textsuperscript{a,***}</td>
<td>12.66±1.67\textsuperscript{a,***}</td>
</tr>
<tr>
<td>Group IV</td>
<td>11.52 ± 1.65\textsuperscript{b,***}</td>
<td>1.89±0.11\textsuperscript{b,***}</td>
<td>7.76±2.64\textsuperscript{b,***}</td>
</tr>
<tr>
<td>Group V</td>
<td>10.98 ± 0.89\textsuperscript{b,***}</td>
<td>1.91±0.24\textsuperscript{b,***}</td>
<td>6.98±1.53\textsuperscript{b,***}</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with Group III. \textsuperscript{***}p< 0.001; ns – non significant.

GSH=μg/mg protein; Vitamin C= μg/mg protein; LPO= nmol of MDA formed/mg protein
5.2.6. Effect of chrysin on enzymatic antioxidants in liver tissue

Table 10 illustrates the effect of chrysin on the levels of enzymatic antioxidants SOD, CAT, GST and GPx in hepatic tissues of control and experimental rats. Diabetic rats showed significant (p<0.001) decrease in the levels of SOD, CAT, GST and GPx in liver tissues. Oral administration of chrysin and glibenclamide treated diabetic rats showed significant (p<0.001) increase in the activities of enzymatic antioxidants to near normal levels. However, chrysin alone treated rats did not show any statistical difference when compared with normal control rats.

Table 10: Effect of chrysin on enzymatic antioxidants in liver tissue of experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>6.36 ± 2.04</td>
<td>6.07± 1.24a**</td>
<td>3.42±0.75a***</td>
<td>6.52± 1.86b***</td>
<td>6.17± 1.43b***</td>
</tr>
<tr>
<td>CAT</td>
<td>31.56± 3.45</td>
<td>35.54±2.45a**</td>
<td>26.45±3.10a***</td>
<td>30.64±1.87b***</td>
<td>31.43±2.36b***</td>
</tr>
<tr>
<td>GST</td>
<td>3.54 ± 1.32</td>
<td>4.12± 1.43a**</td>
<td>2.25±1.43a***</td>
<td>3.04±0.83b***</td>
<td>3.34±1.05b***</td>
</tr>
<tr>
<td>GPx</td>
<td>5.85 ± 1.05</td>
<td>5.06± 0.97a**</td>
<td>4.64±1.00a***</td>
<td>5.55±1.25b***</td>
<td>5.73±0.29b***</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. ***p<0.001; ns – non-significant

SOD: Units are expressed as: 50% inhibition of auto-oxidation of pyrogallol/min/mg protein; CAT: µmol of H₂O₂ consumed/ min/mg protein; GST: µmol of CDNB conjugated min/ mg protein; GPx: µmol of reduced GSH oxidized/min/mg protein.
5.2.7. Effect of chrysin on non-enzymatic antioxidants and lipid peroxidation in liver tissue

The non-enzymatic antioxidants GSH & vitamin C and LPO level in liver tissues are presented in table 11. The level of GSH content and vitamin C in diabetic (Group III) rats decreased (p<0.001) significantly and the level of MDA elevated (p<0.001) significantly. Treatment with chrysin to diabetic rats and glibenclamide treated diabetic rats reverses and brought back to near normal level when compared with normal control rats. No significant differences were observed in chrysin alone treated rats.

Table 11: Effect of chrysin on non-enzymatic antioxidants and LPO in liver tissue of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH</th>
<th>Vitamin C</th>
<th>LPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11.76 ± 2.98</td>
<td>1.68±0.10</td>
<td>6.98±1.34</td>
</tr>
<tr>
<td>Group II</td>
<td>11.77 ± 1.74</td>
<td>1.75±0.38</td>
<td>6.25±1.33</td>
</tr>
<tr>
<td>Group III</td>
<td>9.67 ± 1.65</td>
<td>0.85±0.12</td>
<td>10.22±1.74</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.99 ± 1.34</td>
<td>1.56±0.11</td>
<td>7.76±1.37</td>
</tr>
<tr>
<td>Group V</td>
<td>10.54 ± 0.67</td>
<td>1.63±0.14</td>
<td>6.89±1.99</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant

GSH=μg/mg protein; Vitamin C= μg/mg protein; LPO= nmol of MDA formed/mg protein
5.3. The role of chrysin on glucose homeostasis and insulin signaling in STZ-NA induced experimental T2DM rats

5.3.1. Effect of chrysin on glycolytic enzymes activities in liver tissue

Figure 14 (a) and (b) illustrates the effect of oral administration of chrysin on the activities of hexokinase and pyruvate kinase in liver of control and experimental rats. There were no significant (p<0.001) changes in the levels of glycolytic enzymes treated with chrysin alone. The activities of hexokinase and pyruvate kinase were significantly (p<0.001) decreased in liver tissues of diabetic rats when compared with normal control rats. Chrysin treated diabetic rats similar to glibenclamide treated rats, the altered activities of these enzymes were significantly (p<0.001) inverted to near normal in liver tissue when compared with diabetic control rats.

Figure 14: Effect of chrysin on hexokinase and pyruvate kinase enzymes in hepatic tissue of experimental rats

(a) Hexokinase
(b) Pyruvate kinase

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.3.2. Effect of chrysin on activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase enzymes in liver tissue

The effect of chrysin on the activities of G6PDH and LDH enzymes in hepatic tissue samples were exhibited in figure 15 (a) and (b). Hepatic tissue from chrysin alone treated rats did not exhibit significant difference when compared with normal control rats. Diabetic rats showed significant (p<0.001) decrease in the activity of G6PDH and significant (p<0.001) increase in the level of LDH when compared with normal control rats. Conversely, hepatic tissue of diabetic chrysin treated rats and diabetic glibenclamide treated rats showed significant (p<0.001) increased level of G6PDH enzyme and significantly decreased (p<0.001) in the level of LDH enzyme.

Figure 15: Effect of chrysin on G6PDH enzyme and LDH enzyme in liver tissue of experimental rats

(a) Glucose-6-phosphate dehydrogenase
(b) Lactate dehydrogenase

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.3.3. **Effect of chrysin on gluconeogenic enzymes in liver tissue**

Figure 16 (a) and (b) depicts the activities of glucose-6-phosphatase and fructose-1, 6- bisphosphatase in the liver tissues of control and experimental groups of rats. The liver tissues of diabetic rats showed a significant (p<0.001) increase in the activities of glucose-6-phosphatase and fructose-1,6- bisphosphatase when compared with normal control rats. No significant (p<0.001) variations were found in the rats treated with chrysin alone. However on treatment of chrysin to diabetic rats shows significantly (p<0.001) decreased in the activities of these enzymes restored to near normal level.

**Figure 16: Effect of chrysin on glucose-6-phosphatase and fructose-1,6-bisphosphatase enzyme in liver tissue of experimental rats**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>a***</td>
<td>ns</td>
<td>b***</td>
<td>b***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmoles of Pi liberated/h/mg of protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) **Glucose-6-phosphatase**

(b) **Fructose-1,6-bisphosphatase**

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with Group III. ***p< 0.001; ns – non significant.
5.3.4. Effect of chrysin on glycogenesis and glycogenolysis enzymes in liver tissue

Figure 17 (a) and (b) illustrate the activities of glycogen synthase and glycogen phosphorylase enzymes in hepatic tissue. Chrysin alone treated rats did not exhibit significant differences when compared to normal control rats. Group III rats showed significantly (p<0.001) decreased glycogen synthase and increased glycogen phosphorylase when compared with normal control rats. On chrysin administration to diabetic rats and glibenclamide treated diabetic rats showed significant (p<0.001) increased activities in glycogen synthase and significant increased in the level of glycogen phosphorylation to near normal levels.

Figure 17:  Effect of chrysin on glycogen synthase and glycogen phosphorylase enzyme in liver tissue of experimental rats

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.3.5. Effect of chrysin on glycogen content in liver and skeletal muscle

Figure 18 (a) and (b) showed the effect chrysin on liver and muscle glycogen content of five groups of rats. There were no significant differences in the hepatic and skeletal muscle glycogen content in normal control rats treated with chrysin alone for 28 days. Significantly (p<0.001) reduced glycogen content was observer in both liver and skeletal muscle in group III rats. On oral administration of chrysin to diabetic rats and glibenclamide treated diabetic rats showed significant (p<0.001) increased in glycogen content of both liver and skeletal muscle.

Figure 18: Effect of chrysin on glycogen content in liver and skeletal muscle of experimental rats

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.3.6. Effect of chrysin on glycolytic enzymes activities in renal tissue

The glycolytic enzymes hexokinase and pyruvate kinase enzyme activities in renal tissue of control and experimental were illustrated in the figure 19 (a) and (b). There were no significant (p<0.001) changes in the levels of rats chrysin treated with chrysin alone. The activities of hexokinase and pyruvate kinase were significantly (p<0.001) decreased in renal tissue of diabetic rats when compared with normal control (Group I) rats. On treatment with chrysin as well as glibenclamide, diabetic group of rats showed a significant (p<0.001) increased in glycolytic enzymes which were near normal level when compared with diabetic control rats.

Figure 19: Effect of chrysin on hexokinase and pyruvate kinase enzymes in renal tissue of experimental rats

![Graph showing effect of chrysin on hexokinase and pyruvate kinase enzymes]

(a) Hexokinase  
(b) Pyruvate kinase

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant.
5.3.7. Effect of chrysin on activities of G6PDH and LDH enzymes in renal tissue

Figure 20 (a) and (b) illustrates the effect of chrysin on the activities of HMP shunt enzyme - G6PDH and LDH enzyme in renal tissue of control and experimental rats. There were no significant (p<0.001) changes in the levels of G6PDH and LDH enzyme in rats treated with chrysin alone. Diabetic rats showed significant (p<0.01) increased in the activity of G6PDH and significant (p<0.001) increased in the level of LDH when compared with normal control rats. The activities of hexokinase were significantly (p<0.001) reduced in renal tissues of diabetic rats when compared with normal control rats. Chrysin and glibenclamide treated diabetic rats showed altered activities of these enzymes which were significantly (p<0.001) inverted to near normal in renal tissue when compared with diabetic control rats.

Figure 20: Effect of chrysin on G6PDH enzyme and LDH enzyme in renal tissue of experimental rats

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; **p<0.01; ns – non significant.
5.3.8. Effect of chrysin on gluconeogenic enzymes in renal tissue

Figure 21 (a) and (b) depicts the activities of glucose-6-phosphatase and fructose-1, 6- bisphosphatase in the renal tissues of control and experimental groups of rats. No significant (p<0.001) changes were found in the rats treated with chrysin (Group II) alone. The renal tissues of diabetic control rats showed a significant (p<0.01) increase in the activities of glucose-6-phosphatase and significant increase (p<0.001) in the activities of fructose-1,6- bisphosphatase when compared with normal control rats. However on treatment of chrysin to diabetic rats and glibenclamide treated diabetic rats showed significantly (p<0.001) decreased in the activities of both enzymes and restored to near normal level.

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey's multiple comparison. a – compared with group I; b – compared with group II. ***p< 0.001; **p<0.01; ns – non significant.
5.3.9. Effect of chrysin on the activities of mitochondrial enzymes in liver tissue

Tables 12 showed the activities of TCA cycle enzymes ICDH and SDH and respiratory chain enzyme NADH dehydrogenase respectively. The activities of these enzymes were found to be significantly (p<0.001) lowered on diabetic control rats compared to normal control rats. Diabetic rats treated with chrysin had enhanced activities of mitochondrial enzymes to near normal levels. Conversely, glibenclamide treated rats showed increased activities as like that of chrysin treated diabetic rats.

Table 12: Effect of chrysin on the activities of mitochondrial TCA enzymes and respiratory chain enzyme in liver tissue of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ICDH</th>
<th>SDH</th>
<th>NADH dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>753.66±21.33</td>
<td>30.22±4.56</td>
<td>32.76±5.21</td>
</tr>
<tr>
<td>Group II</td>
<td>765.89±18.99</td>
<td>32.55±5.15</td>
<td>31.78±7.12</td>
</tr>
<tr>
<td>Group III</td>
<td>543.76±22.32</td>
<td>19.89±4.31</td>
<td>16.19±4.87</td>
</tr>
<tr>
<td>Group IV</td>
<td>721.21±23.41</td>
<td>31.13±5.12</td>
<td>28.90±6.78</td>
</tr>
<tr>
<td>Group V</td>
<td>755.21±24.11</td>
<td>30.65±5.72</td>
<td>29.12±5.92</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant

ICDH= nmol of α-ketoglutarate formed/h/mg protein; SDH= nmol of succinate oxidised/min/mg protein; NADH dehydrogenase= nmol of NADH oxidised/min/mg protein
5.3.10. Immunoblot analysis of IRS-1, IRS-2 and GLUT-2 protein expression in liver

The IRS-1, IRS-2 and GLUT-2 protein expression in liver by immunoblot analysis were illustrated in the figure 22 (a) and the densitometric analysis of western blots was given in figure 22 (b). In the liver tissue membrane fraction of diabetic control rats, the IRS-1 and IRS-2 signalling molecules and translocation of GLUT-2 band intensities were significantly (p<0.05) reduced when compared with the band density and relative intensity with β-actin of normal control rats. Oral treatment of chrysin to diabetic (Group IV) and glibenclamide treated diabetic rats showed upregulated protein expression of IRS-1, IRS-2 and translocation of GLUT-2 and increased (p<0.01) significantly in liver. There were no significant differences between the level of intensities in group I and Group II.

Figure 22 (a): Immunoblot of IRS-1, IRS-2 and GLUT-2 in liver tissue. β-actin antibody used as loading control – protein expression
Figure 22 (b): Histogram of IRS-1, IRS-2 and GLUT-2 relative intensity of protein expression in liver compared to β-Actin.

All values are the mean ± SD are accompanied by the number of observations. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison test. ** p<0.01 and *p<0.05 was considered significant

5.3.11. Immunoblot analysis of GLUT-4 protein expression in skeletal muscle and adipose tissue

The GLUT-4 protein expression in skeletal muscle by immunoblot analysis was illustrated in the figure 23 (a) and the densitometric analysis of western blots was given in figure 23 (b). In the skeletal muscle the deficiency of insulin in the diabetic state would reduce the translocation of GLUT-4 from the vesicles to cell membrane. The expression of GLUT-4 was significantly (p<0.01) reduced in diabetic (Group III) rats and after treatment with chrysin to diabetic (Group IV) rats for 28 days resulted enhanced expression significantly (p<0.05) in skeletal muscle. No significant alterations were observed in chrysin alone treated (Group II) rats.
The GLUT-4 protein expression in adipose tissue by immunoblot analysis was illustrated in the figure 23 (c) and the densitometric analysis of western blots was given in figure 23 (d). GLUT-4 protein expression in adipose tissue is down-regulated and significantly (P<0.01) reduced in states of relative insulin deficiency in diabetic rats. On chrysin treatment for 28 days, the expression of GLUT-4 in adipose tissue was increased (p<0.05) significantly resulted enhanced expression in adipocytes.

**Figure 23 (a): Immunoblot analysis of GLUT-4 in skeletal muscle. β-actin antibody used as loading control – protein expression**

**Figure 23 (b): Histogram of GLUT-4 relative intensity of protein expression in skeletal muscle compared to β-Actin.**

All values are the mean ± SD are accompanied by the number of observations. One-way analysis of variance ANOVA followed by Tukey’s multiple comparison test. **p<0.01 and *p<0.05 was considered significant.**
Results

Figure 23 (c): Immunoblot analysis of GLUT-4 in adipose tissue. β-actin antibody used as loading control –protein expression

![Immunoblot analysis of GLUT-4 in adipose tissue](image)

Figure 23 (d): Histogram of GLUT-4 relative intensity of protein expression in adipose tissue compared to β-Actin

![Histogram of GLUT-4 relative intensity](image)

All values are the mean ± SD are accompanied by the number of observations. One-way analysis of variance ANOVA followed by Tukey’s multiple comparison test. ** p<0.01 and *p<0.05 was considered significant.
5.4. The protective role of chrysin on inflammatory biomarkers in STZ-NA induced experimental T2DM rats

5.4.1. Effect of chrysin on protein carbonyl content, nitric oxide and reactive oxygen species in pancreatic tissue

The levels of PCC, NO and ROS in pancreatic tissue were represented in table 13. Diabetic control rats showed significant (p<0.001) increased in the levels of PCC, NO and ROS when compared with normal control rats. Oral administration of chrysin treated diabetic rats for 28 days showed significant (p<0.001) reduction in PCC, NO and ROS in pancreatic tissue when compared with diabetic rats and homogeneously significant (p<0.001) decrease was observed in glibenclamide treated diabetic rats. However, chrysin treated rats did not show any adverse effects when compared with control group of rats.

Table 13: Effect of chrysin on PCC, NO and ROS in pancreatic tissue of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCC</th>
<th>NO</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12.57±1.23</td>
<td>6.87±0.8</td>
<td>42.06±5.6</td>
</tr>
<tr>
<td>Group II</td>
<td>15.56±3.4</td>
<td>5.87±1.1</td>
<td>36.04±6.7</td>
</tr>
<tr>
<td>Group III</td>
<td>21.43±3.5</td>
<td>11.23±0.9</td>
<td>78.09±7.88</td>
</tr>
<tr>
<td>Group IV</td>
<td>17.42±2.53</td>
<td>6.81±0.11</td>
<td>50.67±7.83</td>
</tr>
<tr>
<td>Group V</td>
<td>13.51±3.44</td>
<td>7.43±0.71</td>
<td>48.90±8.94</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant

PCC= nmoles/mg of protein; NO= nmoles/mg protein; ROS= nmoles/mg protein
5.4.2. Effect of chrysin on protein carbonyl content, nitric oxide and reactive oxygen species in liver tissue

Table 14 represented the levels of PCC, NO and ROS in liver tissue. In diabetic control rats there was significant increase (p<0.001) in the levels of PCC, NO and ROS when compared to normal control (p<0.001) rats. Treatment with chrysin and glibenclamide for 28 days showed significant (p<0.001) decrease in PCC, NO and ROS in liver tissue when compared with diabetic control rats. No significant alterations were observed in chrysin treated rats.

Table 14: Effect of chrysin on PCC, NO and ROS in liver tissue of experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCC</th>
<th>NO</th>
<th>ROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11.99±2.31</td>
<td>7.67±1.05</td>
<td>32.33±3.45</td>
</tr>
<tr>
<td>Group II</td>
<td>13.54±1.4</td>
<td>6.56±2.1</td>
<td>32.56±5.84</td>
</tr>
<tr>
<td>Group III</td>
<td>23.51±2.5</td>
<td>12.43±0.6</td>
<td>45.32±5.96</td>
</tr>
<tr>
<td>Group IV</td>
<td>13.52±1.1</td>
<td>8.54±1.67</td>
<td>35.12±6.38</td>
</tr>
<tr>
<td>Group V</td>
<td>13.42±1.88</td>
<td>8.63±1.41</td>
<td>32.43±4.65</td>
</tr>
</tbody>
</table>

Values were given as mean ± SD for six rats in each group. Data analysis was performed by one-way ANOVA followed by Tukey’s multiple comparison. a – compared with group I; b – compared with group III. ***p< 0.001; ns – non significant

PCC= nmoles/mg of protein; NO= nmoles/mg protein; ROS= nmoles/mg protein
5.4.3. **Reverse transcription (RT)-PCR analysis of mRNA transcript levels**

mRNA transcripts of TNF-α, IL-6, IL-1β, NF-κB and iNOS were generated by RT-PCR and the transcript levels in pancreatic tissues of the different groups compared in figures 24 (a), (b) and hepatic tissue compared of different groups compared in figure 25 (a) and (b). The transcript level of the genes in pancreatic and hepatic tissues of diabetic (Group III) rats was found to be significantly (P<0.01) higher than that of normal control (Group I) rats, while the level of these transcripts in pancreatic and hepatic tissue samples of chrysin treated diabetic (Group IV) rats was significantly (P<0.05) lower than that of pancreatic and hepatic tissue samples of diabetic control rats.

**Figure 24 (a): Reverse transcription-PCR analysis of TNF-α, IL-6, IL-1β, NF-κB and iNOS mRNA expression in pancreatic tissue samples and β-actin (internal control) in an ethidium bromide-stained agarose gel**
Figure 24 (b): Histogram of mRNA transcript levels of TNF-α, IL-6, IL-1β, NF-κB and iNOS in pancreatic tissue samples

All values are the mean ± SD are accompanied by the number of observations. One-way analysis of variance ANOVA followed by Tukey’s multiple comparison test. **p<0.01 and *p<0.05 was considered significant

Figure 25 (a): Reverse transcription-PCR analysis of TNF-α, IL-6, IL-1β, NF-κB and iNOS mRNA expression in hepatic tissue samples and β-actin (internal control) in an ethidium bromide-stained agarose gel
Figure 25 (b): Histogram of mRNA transcript levels of TNF-α, IL-6, IL-1β, NF-κB and iNOS in hepatic tissue samples

All values are the mean ± SD are accompanied by the number of observations. One-way analysis of variance ANOVA followed by Tukey’s multiple comparison test. ** p<0.01 and *p<0.05 was considered significant.
6.1. Hypoglycemic effect of chrys in on STZ-NA induced experimental T2DM rats

STZ is a nitrosourea analogue and selective β-cell genotoxicant has been extensively used to induce selective destruction of β-cells of the pancreas which leads to decrease in insulin secretion (Frode and Medeiros 2008). The extensive activation of poly-ADP ribose synthetase on STZ treatment results in rapid depletion of cellular NAD$^+$ which further leads to β-cell necrosis. The administration of NA, a poly-ADP ribose synthetase inhibitor, protects the β-cells by minimizing the decline in NAD$^+$ and thereby improve pancreatic insulin secretion nearly upto 40%. Therefore used as an ideal model to induce T2DM in experimental rats (Shima et al., 1998). As a consequent, the rate of insulin secretion and concentration of plasma insulin is diminished significantly that ultimately results in a clinical condition known as hyperglycemia. The STZ-NA induced diabetes rat model has been characterized by the clinical conditions of non-obese, constant hyperglycemia, glucose intolerance similar to T2DM in humans, and also used to study short as well as long term effects of drugs and natural compounds on diabetic complications (Szkudelski, 2012; Masiello et al., 1998). Hence, in the present study STZ-NA induction has been selected to induce T2DM in rats and to evaluate the antihyperglycemic potential of chrys in.

Sulphonylureas act by increasing insulin secretion from the pancreatic β-cells and glibenclamide in a sulfa drug which has been used as a standard antihyperglycemic drug to compare the antidiabetic efficacy of chrys in. Glibenclamide is involved in the stimulation of insulin secretion from β-cells of the pancreas by activating ATP-sensitive potassium (K$^{-\text{ATP}}$) channels in the plasma membrane (Ashcroft and Ashcroft
1992). This causes cell membrane depolarization, opening voltage-dependent calcium channels and resulted increased intracellular calcium in the β-cell and subsequent stimulation of insulin release (Serrano-Martin et al., 2006).

In T2DM, insulin insufficiency leads to the development of hyperglycemia with dysregulated changes in glucose homeostasis. The fundamental mechanism underlying hyperglycemia in DM suggests that increased rate of hepatic glycogenolysis and gluconeogenesis with decreased utilization of glucose by other tissues (Paulsen, 1973). Characteristic symptoms in diabetes such as increased water intake, food intake and severe loss of body weight supports the accumulation of glucose in the blood and excess excretion of glucose in urine (Punithavathi et al., 2008). In STZ-NA induced diabetic rats, increased food consumption and decreased body weight were observed which could be due to excessive break-down of tissue proteins (Chatterjea & Shinde 2002). Decrease in body weight of the diabetic rats could be due to dehydration and catabolism of fats and proteins (Hakim et al., 1997) which leads to muscle wasting and thus causing reduction in weight gain by diabetic rats (Rajkumar et al., 1991). However, diabetic rats treated with chrysin for 28 days showed improvement in body weight with normal food and water consumption.

OGTT is a more sensitive measure of early abnormalities in glucose homeostasis (Ceriello, 2005). The lower boundaries for the classification of impaired glucose tolerance was established to reflect both the increased risk of developing T2DM and cardiovascular disease risk even at these lower levels of perturbed glucose metabolism (Khaw et al., 2001). An increase in OGTT blood glucose value noticed in diabetic rats remained high even after 120 min. In contrast, on chrysin treatment the blood glucose level lowered at the end of 60min and even lowered at the end of 120min. Chrysin treated diabetic rats showed enhanced glucose utilization and therefore significantly reduced blood glucose level following OGTT.
The administration of chrysin effectively prevented the increase in the blood glucose level without causing a hypoglycemic state, probably due to the insulin secretion from remnant β-cells. The reduction was also reflected in urine sugar level and the findings were agreed with Lukacinova et al., 2008. Glycosuria is the common symptom of diabetes and it arises due to the increase in blood glucose above 250 mg/dl in diabetic animals (Stanely et al., 2004). However, diminished insulin response in diabetes mellitus retards protein synthesis and stimulates protein degradation, and thus decreasing the synthesis of hemoglobin (Sheela and Augusti 1992). During diabetes the excess glucose present in blood reacts with haemoglobin and glycosylation occurs leading to the formation of glycosylated haemoglobin (Ahmed and Urooj, 2009) which was a standard marker for ambient glycemia and indicates the degree of protein glycation (Koenig et al., 1976). However, glycosylated haemoglobin reduced in chrysin treated diabetic rats which may be due to hypoglycemic effect of chrysin.

The protective nature of chrysin in experimental rats was further evidenced from histological study. STZ is known to induce chemical diabetes by selective destruction of pancreatic β-cells through three processes viz: DNA alkylation, nitric oxide production and free radical generation, leading to a total lack or deprived insulin production and chronic hyperglycaemia (Das et al., 1996; Szkudelski, 2001). In the present study, islets of normal rat showed normal architecture whereas pancreatic tissue of diabetic induced rats depicted profound distortion in its structural organization, degranulation of β-cells and depletion of islets (Bora et al., 1985). The present study results support the possible role of chrysin in regeneration of β-cells and thus in the treatment of diabetes. Improvement in the histoarchitecture and cell mass of islets in chrysin treated diabetic rats might be due to the regeneration of the damaged β-cells and protection of new β-cells in the pancreas. The antioxidant potential of chrysin may
quench to control the population of ROS generated by STZ, in pancreatic tissues of the rats, thereby allowing the regeneration of pancreatic islets cells.

The liver is the prime organ of the body preoccupied with the function of glucose homeostasis and biotransformation of xenobiotics / drugs (Champe et al., 2005). Satav and Katyara, 2004 studied the effect of STZ-induced diabetes on the oxidative energy metabolism in rat liver mitochondria and found reduction in respiratory activity. Lukivskaye et al., 2007 related the liver pathological changes in alloxan diabetic rats to the mitochondrial abnormalities. Many studies suggested that liver mitochondrial dysfunction in diabetes can be related to enhanced oxidative stress in diabetic animals (Kucharska et al., 2000; Bukker et al., 2000). The major pathological alterations observed in liver of diabetic rats were necroticed and inflammatory cell infiltration which may be due to β-cell degeneration (Ong and Khoo, 2000). In the present study, STZ-NA induced rats exhibited lesions, congestions and dilation with mild inflammatory cell infiltrate and distortion of hepatocytes. However, chrysin treatment to diabetic group of rats demonstrated the distinct improvement in the hepatocytes and regeneration of hepatic tissue.

Diabetic nephropathy is one of the most serious complications in DM and has been the most common cause of end-stage renal failure among patients undergoing chronic hemodialysis therapy (Nakai et al., 2005). In the present study STZ-NA induced rats showed, progressive widespread tubular necrosis with loss of brush border in most part of the kidney sections. These finding agree with the findings of Kim et al., 2008 and Renno et al., 2008. All these pathological changes were reduced and kidney showed normal architecture of glomerular and tubular cells without any inflammation, on administrations to diabetic rats with chrysin. The various segments of kidney tubules were well preserved without inflammation or congestion or necrosis indicating the non-toxic effects of chrysin.
6.2. Effect of chrysin on hyperglycemia mediated oxidative stress in STZ-NA induced experimental T2DM rats

Liver is the vital metabolic organ plays a major role in the regulation of blood glucose and is responsible for disposal of one third of oral glucose load. Several hormones, including insulin, glucagon, growth hormone, cortisol, and catecholamines contribute to the regulation of glucose metabolism in liver (Cotrozzi et al., 1997). AST is the cytosolic enzymes associated with the conversion of amino acids to ketoacids and is considered as the marker enzyme to assess tissue damage. ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate impairment in insulin signalling followed by tissue damage (O’Brien and Granner, 1991). ALP acts as a marker of biliary function and cholestasis. It is hypothesized that elevation of AST, ALT and ALP are considered as predictors of diabetes and leak from the cytosol into bloodstream as a consequence of damage in hepatic tissue (El-Demerdash et al., 2005). On chrysin treatment the diabetic rats significantly reduced the serum hepatic biomarkers suggesting the protective effect of chrysin against hyperglycemia mediated oxidative stress.

Stepwise increase in three nitrogenous constituents of blood namely urea, uric acid and creatinine are believed to reflect a deteriorating kidney function which is considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). By the removal of these metabolic wastes, maintains the optimum balance in the body fluids. Urea is the major end product of protein metabolism and uric acid is the major end product of purines metabolism whereas, creatinine is endogenously produced by creatinine metabolism and released into body fluids and their clearance is measured as an indicator of glomerular filtration rate (Burtis and Ashwood, 1996). In T2DM, renal damage occurs due to abnormal glucose regulation, elevated blood glucose, glycosylated protein and oxidative stress (Aurell and Bjorck, 1992). The present study indicated significant reduction in the levels of serum urea, uric acid and creatinine after chrysin treatment indicating the renoprotective role of chrysin in diabetic rats.
A variety of alterations in lipid metabolism and its dysregulation occurs due to insulin deficiency which further leads to the accumulation of serum lipids (Rajalingam et al., 1993). Abnormal lipid profile is one of the common problems accompanied with T2DM (Taskiman et al., 1987). The elevated serum levels of TC, TGs, LDL, VLDL and reduced HDL cholesterol in diabetic condition, cause to be a risk factor for development of microvascular complication, to other coronary heart disease like atherosclerosis (Singh et al., 2010). STZ-NA induced diabetes also developed hyperlipidemia which is in agreement with other previous observations (Akram et al., 2014; Pari and Murugan, 2007). Cholesterol and triglycerides are transported in the blood by combinations of lipids and proteins called lipoproteins. Insulin deficiency or insulin resistance may be responsible for dyslipidemia. Since insulin is necessary to activate lipoprotein lipase which hydrolyse the triglycerides in lipoprotein. Hence, when insulin is low lipoprotein lipase is low and hence triglyceride level is increased in diabetic rats. Insulin has stimulatory effect on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles (Guoyan, 1992). The reduction in serum TC, TGs, LDL and VLDL level and increased in HDL level in chrysin treated diabetic ratsmay be due to the hypolipidemic effect of chrysin.

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems which results due to increased free radical formation or reduced activity of antioxidant defences or both. Oxidative stress in the pathogenesis of DM is related not only to oxygen free-radical generation and lipid peroxides but also non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism and alteration in antioxidant enzymes (Ahmed, 2005). West, (2000) postulated that the chronic hyperglycemia in DM leads to an increased production of ROS, which possibly results in depletion of certain enzymatic and non-enzymatic antioxidants.
Enzymatic antioxidants namely SOD, CAT, GST and GPx and non-enzymatic antioxidants such as GSH and vitamins C play an essential role in preventing free radical induced tissue damage (Kanchana et al., 2011). The efficiency of these enzymes are altered in T2DM and therefore, it play a crucial role in pathogenesis of DM. The first line defense against ROS is regulated by antioxidant enzymes such as SOD, CAT and GPx by scavenging the toxic intermediate on oxidation (Babujanarthanam et al., 2011). SOD scavenges the free superoxide radicals whereas CAT and GPx catalyze the decomposition of H$_2$O$_2$ to H$_2$O and oxygen (Irshad and Chaudhuri, 2002). GST, a glutathione dependent enzyme, protects cells from ROS by utilizing a wide variety of products of oxidative stress as substrates (Bekris et al., 2005). GSH is a ubiquitous tripeptide thiol, an important intracellular metabolite. It acts as an antioxidant and provides secondary line of defence against intracellular free radicals and peroxides generated under oxidative stress (Rashid et al., 2013). Vitamin C is an excellent hydrophilic antioxidant; with peroxyl radical scavenging activity (Atanasiu et al., 1998). The level of enzymatic antioxidants SOD, CAT, GST and GPx and non enzymatic antioxidants GSH and vitamin C were diminished in all the tissue mainly pancreas and liver of diabetic rats (Alejandro et al., 2013) were also agreed with our results. However, administration of chrysin significantly increased the activities of both enzymatic and non enzymatic antioxidant enzymes in both pancreas and liver tissue as like that of glibenclamide treated rats.

LPO is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction (Pari and Latha, 2005). MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage (Ohkawa et al., 1979). The extent of LPO was measured as TBARS has been reported to raise in diabetes (Rajasekaran et al., 2005) and the raised level of TBARS on STZ-
NA administration in pancreas and liver suggests increased production of free radicals such as superoxide (O$_2^{•−}$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH•) (Kakkar et al., 1995). In this study lower levels of pancreatic and hepatic antioxidant enzymes and increased LPO in diabetic groups were observed when compared with normal control while in chrysin treated diabetic group oxidativestress was found to be reduced by the imbalance between the generation of ROS and scavenging enzymes. Therefore, treatment with chrysin increasing the activity of antioxidant enzymes and thereby controlling hyperglycemia mediated oxidative stress.

6.3. The role of chrysin on glucose homeostasis and insulin signaling in STZ-NA induced experimental T2DM rats

Liver is the primary organ for endogenous glucose production with a minor contribution from the kidney which produces glucose either from gluconeogenesis or via glycogenolysis (Prasath and Subramanian, 2011; Meyer et al., 2004). Skeletal muscle constitutes up to 50% of total body mass and approximately 80% of blood glucose is metabolized by insulin-sensitive tissues such as skeletal muscle and adipose tissue (Rennie et al., 2004). Defects in carbohydrate metabolism and consistent efforts of the physiological systems to overcome the imbalanced carbohydrate metabolism pose an over exertion on the endocrine system, which leads to the deterioration of endocrine control (Cheplick et al., 2010). Continuous deterioration of endocrine control aggravates the metabolic disturbances by altering carbohydrate metabolic enzymes and thereby leads primarily to hyperglycemia (Tiwari and Madhusudana, 2002). The insulin insensitivity and insulin deficiency in T2DM leads to decreased utilization of blood glucose utilization by the liver, muscles and adipose tissue with increased hepatic glucose production (McGarry, 1992). Insulin regulates glucose homeostasis by modulating the uptake and utilization of glucose in target organs such as liver, kidney,
skeletal muscle and adipose tissue by controlling the activities of numerous metabolic enzymes (Seifter and England, 1982).

HK is an isoenzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate thus playing a crucial function in tissue intermediary metabolism. Reduced activity of HK in diabetic rats leads to impairment of oxidation of glucose via glycolysis, thus results in hyperglycemia with decreased ATP production (Wilson, 1995). Therefore, a raise in the activity of hexokinase can increase glycolysis and thus improve energy production. The markedly decreased level of insulin in the STZ-NA induced diabetic animals ultimately leads to the impairment in the activity of HK, since insulin deficiency is a hallmark of diabetes (Laakso et al., 1995). However, chrysin administration enhances glucose metabolism both in liver and kidney and promotes overall glucose homeostasis by increasing the activity of HK.

PK is key regulatory glycolytic enzyme in glycolytic pathway that catalyzes the conversion of phosphoenolpyruvate to pyruvate (Yamada and Noguchi, 1999). Hence, the observed decline in the activity of PK in the liver and kidney of STZ-NA induced diabetic rats is due to the reduced glycolysis and increased gluconeogenesis (Taylor and Agius, 1998). The altered activity of PK in diabetes expected to reduce the metabolism of glucose and ATP production. On treatment with chrysin the diabetic rats showed high PK activity in both hepatic and renal tissue, thus improving energy production.

G6PDH, a “housekeeping” enzyme, catalyzes the first and rate-limiting step of the hexose monophosphate (HMP) shunt and produces NADPH, which is essential for the maintenance of reduced glutathione and reductive biosynthesis (Mayes, 2000). The reduction of G6PDH activity in liver which obstruct glucose utilization through pentose phosphate pathway as this enzyme activity is regulated by insulin (Ugochukwu and Babady, 2003). In this study, the activity of G6PDH was significantly decreased in
STZ-NA induced diabetic rats in liver and kidney that might be due to decrease in GSH level and the activity of the enzyme is raised to near normal after the treatment of chrysin.

LDH is a terminal glycolytic enzyme that play an indispensable role in the inter-conversion of pyruvate to lactate to yield energy under anaerobic conditions (Kavanagh et al., 2004; Ainscow et al., 1999). This excessive pyruvate is converted to lactate and therefore the activity of LDH may be increased due to less insulin availability in diabetes (Ramachandran et al., 2003). Elevated LDH levels observed in the diabetic animals was associated with impaired glucose-stimulated insulin secretion. On treatment with chrysin, the diabetic rats LDH activity was reversed in both hepatic and renal tissue to near normally.

G6Pase, a key enzyme involved in the regulation of blood glucose concentration, is expressed mainly in the liver and kidney and it plays a major role in gluconeogenesis under prolonged fasting or starvation (Bouche et al., 2004). G6Pase, is mainly found as an integral protein in the lumen of the endoplasmic reticulum which catalyzes the dephosphorylation of G6P to glucose and phosphate. It has been involved in both glycogenolysis and gluconeogenesis (Roden and Bernroider, 2003). Our findings were in consistent with previous reports on this gluconeogenic enzymes which states marked increase in the G6Pase activity in diabetes (Jang et al., 2010). Chrysin treated diabetic rats decreases the activity of G6Pase either through metabolic activation of glycolysis or inhibition of gluconeogenesis in both liver and kidney.

F1, 6BPase is an important rate limiting enzyme that catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate, in gluconeogenic pathway and its activity promotes the release of hepatic glucose into circulation under diabetic condition (Pilkis and Claus, 1991). The diabetic rats treated with chrysin a marked decrease in F1,6 BPase was noted which further supports the regulatory role of chrysin.
Glycogen is an intracellular storage form of glucose in liver and muscle and directly reflected with insulin action. Insulin regulates glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase enzyme (Pederson et al., 2005). Glycogen synthase, a crucial and rate-limiting enzyme in tissues non-oxidative glucose disposal, catalyzes the transfer of glucose from UDP-glucose to glycogen (Parker et al., 2004). Glycogen phosphorylase is the rate-limiting enzymes in glycogenolysis. Its activity is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose (Bollen et al., 1998). Glycogen levels of liver and skeletal muscle were decreased due to the deficiency of insulin and enhanced glycogen phosphorylase whereas conversely, reduced glycogen synthase activity. While on chrysin treatment the diabetic rats improved liver and muscle glycogen content which could be due to stimulated effect of insulin.

Mitochondria undergo fragmentation with a concomitant increase in ROS formation after exposure to high glucose concentrations. Reduction in the activities of mitochondrial enzymes ICDH, SDH and NADH dehydrogenase indicated that the mitochondrial oxidative phosphorylation was operating lower level in the liver (Yu et al., 2006). In the present study, we observed the decreased activities of mitochondrial enzymes such as ICDH, SDH and NADH dehydrogenase in the STZ-NA induced diabetic rats. This may be correlated with the increased levels of LPO and decreased levels of antioxidants in STZ-NA induced diabetic rats. ICDH is also involved in the supply of NADPH needed for GSH production which acts against cytosolic and mitochondrial oxidative damage (Bailey and Cunningham, 2002). The decline in activity of mitochondrial enzymes may result in the perturbation of the balance between oxidants and antioxidants and subsequently lead to a pro-oxidant condition. Chrysin treated diabetic rats shows increased in the activities of mitochondrial enzymes, probably by recovering the mitochondrial antioxidant defence system and also scavenge ROS formation.
Insulin resistance in liver was depicted with reduced expression of both IRS-1 and IRS-2 (Taniguchi and Kahn, 2005). IRS proteins are critical link in hepatic insulin signalling, autophosphorylation of the IR by insulin results in the recruitment and activation of intracellular downstream signalling molecules and leads to glucose uptake and various other biological effects (Saltiel and Pessin, 2002; White and Kahn, 1994). IRS-1 and IRS-2 are the major substrates of insulin receptor kinase and has many tyrosine phosphorylation sites, which provide binding sites for many kinases including phosphatidylinositol-3-kinase (PI3-kinase). Tyrosine phosphorylation of IRS leads to the activation of GLUT. Impairment in IRS-1 and IRS-2 phosphorylation and therefore a lack of downstream mediated insulin signalling occurs in diabetes. Decreased level of IRS proteins in liver may be due to insulin insufficiency and hepatic insulin resistance (Sun et al., 2002). Liver has a distinct glucose transporter GLUT-2 whereas, skeletal muscle and adipose tissue express another type of glucose transporter is GLUT-4 (Scheepers et al., 2003). GLUT-2 is the major glucose transporter protein involved in transport of glucose across hepatocytes, depending upon the insulin stimuli (Slieker et al., 1992) and it has been found to be downregulated in STZ-NA induced diabetic rats.

GLUT-4, a member of the GLUT family, is mainly expressed in skeletal muscle and adipose tissues and plays a major role in glucose transport in these target tissues. Insulin stimulated translocation of GLUT-4 from the intracellular pool to the plasma membrane (Shepherd and Kahn, 1999). The GLUT-4 expression in skeletal muscle is down-regulated due to relative insulin deficiency in STZ-NA induced diabetes. The decreased GLUT-4 levels in skeletal muscle and adipose tissue is essentially one of the main reasons of hyperglycemia in the STZ-NA diabetic state, which leads to decreased uptake of glucose. Restoration of GLUT-4 levels on chrysin treatment enhance the uptake of glucose in the skeletal muscles and adipose tissue, thus exhibits hypoglycemic effect in STZ-NA induced diabetic rats. After treatment with chrysin GLUT-4 protein expression are restored to near normal level in both skeletal muscle and adipose tissue.
6.4. **Protective role of chrysin on inflammatory biomarkers in STZ-NA induced experimental T2DM rats**

Hyperglycemia mediated oxidative stress results in the production and release of pro-inflammatory mediators like cytokines and chemokines which are believed to play a pivotal role in the pathogenesis of T2DM (Herder et al., 2009). PCC is a stable oxidative stress marker for T2DM and increased level was observed in cells and tissues (Dayanand et al., 2012). Under diabetic conditions, electron transport chain is activated, which leads to production of larger amounts of ROS (Santini et al., 1997). Increased production of ROS resulting in tissue damage that is the most instances assessed by the measurement of protein carbonyl. Disorder of physiological signaling functions of ROS superoxide and hydrogen peroxide and nitric oxide is an important feature of T2DM (Afanas, 2011). Studies suggest that ROS and NO are generated in both cytokine-stimulated and STZ-treated pancreatic β-cells mediate the deleterious effects of proinflammatory cytokines or STZ on β-cells dysfunction and destruction (Rabinovitch et al., 1998). As a toxic inflammatory effector molecule on the impairment of islet β-cells, NO is mainly produced by iNOS in inflammatory macrophages and the inflamed pancreatic β-cells themselves (Stamler et al., 1992). Moreover, it has been suggested that NO produced spontaneously from STZ may take part in pancreatic β-cell damage because STZ has a nitrosourea group in its molecule. NO mediates cytotoxicity through several routes: NO rapidly reacts with the superoxide anion, which results in the formation of peroxynitrite and hydroxyl radicals, both powerful oxidants, and excess NO generated in cells may inhibit mitochondrial metabolism and contribute to protein modification and DNA cleavage, any one of which could lead to β-cells death (Bartosz, 1996).

In the present study, oral administration of chrysin alleviates PCC, ROS and NO production to near normal levels. The detonated production of free radicals, during chronic hyperglycemia evoked oxidative stress and was restored to normal level on treatment with chrysin; DM is responsible for the overproduction of proinflammatory
Discussion

cytokines and perpetuated its deleterious effects on pancreatic islets. The proinflammatory cytokines released from the pancreatic β-cells such as IL-1β, IL-6 and TNF-α and proinflammatory transcription factor, NF-κB and consequent synthesis of NO radical have been implicated in the pathogenesis and progression of diabetes mellitus (Leung and Chan, 2009). NF-κB is a pro-inflammatory “master switch” that regulates the expression of an array of genes encoding cytokines, including TNF-α and IL-1β, IL-6. Hence, it leads to enhanced production of these cytokines. Although IL-1β increases the generation of reactive species, IL-6 exacerbates the insulin resistance and TNF-α aggravates the destruction of islets, which plays a significant role in dysfunction of β-cells under cytokine mediated damage (Lenzen, 2008).

Supraphysiological glucose also favors the augmented expression of iNOS in inflammatory macrophages through the activation of NF-κB that ultimately results in the elevated production of NO. In conjunction with oxidative stress, the levels of the proinflammatory cytokines, TNF-α, IL-6, IL-1β were elevated (Jain et al., 2003). IL-6 is a pro-inflammatory cytokine and increased in T2DM insulin resistance (Straub et al., 2008), impaired glucose tolerance and T2DM (Muller et al., 2002). Increases in IL-6 lead to a reduction in IRS-1 tyrosine phosphorylation, a decreased association between the PI-3 kinase and IRS-1 and an inhibition of insulin-dependent activation (Bastard et al., 2000; Senn et al., 2002). In the present investigation, we have observed the elevated levels of NF-κB, IL-1β, IL-6, TNF-α and iNOS in STZ-NA induced diabetic rats in pancreas and liver tissue. Administration of chrysin suppresses the mRNA expression of proinflammatory cytokines in pancreatic and liver tissue of rats. Thus the results of the present investigation suggested that chrysin might possess significant anti-inflammatory potential, thereby it might prevent the production of noxious mediators involved in the development of T2DM.