RENO PROTECTIVE EFFECT OF CHRY SIN (5,7 DIHYDROXY FLAVONE) IN STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY IN RATS

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ABSTRACT

The present study was designed to investigate the effect of chrysin in experimentally induced diabetic nephropathy in rats. Wistar male albino rats were divided into four groups. Control rats (Group-I) received dimethyl sulfoxide, diabetic rats (Group-II) received STZ (50mg/kg bwt), (Group-III) rats received chrysin (20mg/kg bwt) and (Group-IV) rats received STZ (50mg/kg bwt) and chrysin 20mg/kg bwt. Blood and urine samples were collected every four weeks to measure blood glucose, urea, serum creatinine, protein, urine urea, creatinine, protein and glomerular filtration rate was determined. The levels of blood glucose, urea, serum creatinine, total urinary protein, urine urea, creatinine, protein and glomerular filtration rate were increased and Glomerular filtration rate was significantly (p<0.001) reduced in diabetic nephropathy rats. Co-administration of chrysin to STZ induced diabetic nephropathy rats significantly (p<0.001) reduced the levels of blood glucose, urea, serum creatinine, urinary glucose, urea, creatinine, protein and elevated the level of Glomerular filtration rate. These results suggested that chrysin has renoprotective effect against STZ induced diabetic nephropathy in rats.

Keywords: Streptozotocin, Diabetic nephropathy, Hyperglycemia, Glomerular filtration rate, Chrysin.

INTRODUCTION

Diabetic Nephropathy is one of the most serious complications of diabetes and common cause of end-stage renal failure. At present 40% of the patients with type-11 diabetes suffer diabetic kidney diseases. The characteristic features of these diseases are persistent albuminuria, a decline in glomerular filtration rate and structural alterations such as thickened glomerular basement membrane and progressive accumulation of extracellular matrix protein in the glomerular mesangium.

The involvement of various derangements associated with diabetes can be considered in the development of diabetic nephropathy. Among them hyperglycemia play an important role in renal injury. The magnitude of hyperglycemia correlates with the functional and structural changes of diabetic nephropathy. Clinically, strict glycemic control inhibits both the functional decline in GFR and the formation of characteristic structural lesions. The restoration of glycemia reverses structural changes. Exposure to high glucose causes an increase in matrix protein generation and cell cycle arrest by cultured cells, development of novel therapeutic agents inhibiting the aforementioned factors is of particular interest as they represent potential treatments for the prevention of diabetic complications.

Several clinical trials and studies have shown that improved glycemic control is strongly associated with decreased development or regression of diabetic complications in both type1 and typeII diabetic mellitus and glomerulosclerosis with other clinical or pathologic evidence that sclerosis is attributable to diabetic nephropathy. Flavonoids constitute the largest and most important group of polyphenolic compounds in plants. It is now widely accepted that dietary polyphenolics may play an important role in protecting the body against chronic diseases, such as cancer, cardiovascular diseases and diabetes mellitus.

Chrysin (5, 7 dihydroxy flavone) is a polyphenolic compound derived from species like passiflora, pelargonium and pinaceae. It is naturally present in honey, plant extracts, propolis and pine wood. Chrysin exhibits a strong complexing activity for clinical and pathologic evidence that sclerosis is attributable to diabetic nephropathy.

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Chrysin exhibits a strong complexing activity for clinical and therapeutic applications in various diseases. Like other flavonoids, chrysin exhibits many beneficial effects and pharmacological activities such as an anti-inflammatory, antioxidant, antihypertensive, antidiabetogenic and anticancer. Chrysin also has the potency for clinical and the therapeutic application against the physiological and biochemical effects of aging. In vivo studies have indicated that chrysin offers protection against oxidative stress mediated ethanol-induced liver injury and also suggests the chemoprotective effects on breast and colon cancers. Chrysin acts as a hepatoprotective and antioxidant agent against D-galactosamine-induced hepatotoxicity. The present study was undertaken to evaluate the renoprotective effect of chrysin in Streptozotocin induced diabetic nephropathy in rats.

MATERIALS AND METHODS

Animals

Healthy Wistar male albino rats, weighing 180-200g were obtained from Saveetha University, Chennai, India and maintained in a diurnal light and dark cycle of 12h each. Rats were fed with standard food pellets and given access to water ad libitum. Rats were left for one week for acclimatized before starting the study. The experimental designs were approved by the Institutional Ethical Committee of the Saveetha University, Chennai (009/2010/CPSEA).

Chemicals

Streptozotocin (STZ) and Chrysin were purchased from Sigma Chemicals Co (St. Louis, Mo, USA). All other chemicals used in this study were of analytical grade and obtained from SRL Chemicals, Mumbai, India.

Experimental induction of diabetic mellitus

Diabetes was induced by single injection of STZ at a dose of 50mg dissolved in 0.1M citrate buffer (PH 4.5)/kg body weight, intra peritoneally, after 16h fasting. After injection the animals were free access to food and water. After 4h the animals were given with 10% glucose in their drinking water for the first 24h to counter any initial hypoglycemia. 72h after STZ injection diabetes was confirmed in rats by blood sugar level greater than 250mg/dl. Animals with blood glucose levels greater than 250mg/dl was considered for further study. Blood samples were collected every four weeks from orbital plexus by prickling a needle under ketamine anaesthesia. Blood glucose was determined by using o-toluidine reagent.

Experimental designs

Experimental animals were divided into four groups and each group consisting of six animals.

Group 1: Rats received Dimethyl Sulphoxide (1% DMSO) as vehicle i.p for 20 weeks and referred as positive control rats

Group 11: Rats were administered i.p a single dose of STZ 50mg dissolved in 0.1M citrate buffer PH 4.5/kg body weight and served as a diabetic rats.
Group 11: Rats were treated with chrysin 20 mg dissolved in 1% DMSO/kg body weight i.p for 20 weeks to assess the toxicity if any induced by chrysin and rats were referred as drug control.

Group IV: Rats were received STZ 50mg/kg body weight (as in Group 11) along with Chrysin 20mg/kg body weight (as in Group 11) and rats were referred as treated rats.

**Sample collection**

The change in body weight and level of glucose in all groups of rats were recorded at regular intervals throughout the study. During the experimental period the animals were placed in individual metabolic cages every 4 weeks and 24h urine samples were collected for the measurement of urea, creatinine, total protein and creatinine clearance. Rats had free access to water while in metabolic cages.

**Biochemical Parameters**

Nephropathy was evaluated by estimating blood urea and urinary protein. Further creatinine clearance was also determined as a measure of glomerular filtration rate (GFR)²⁴. Creatinine clearance was assessed from the urinary and serum creatinine and expressed as ml/min/kg body weight. Blood and urinary urea was estimated by diacetyl monooxime method²⁵, serum and urinary creatinine were measured by alkaline picrate method²⁶. Urinary protein was quantified by Lowry’s method²⁷. Glycosylated Hemoglobin was determined by the method of Nayak and Pattabiraman²⁸ and plasma insulin was estimated by ELISA kit (for rats) supplied by Lincoplex Ltd. (USA) method.

**Statistical Analysis**

The values are expressed as mean ± SD for six animals in each group. Differences between groups were assessed by One-way analysis of variance (ANOVA) using SPSS software package for windows. Post hoc testing performed for inter-group comparisons using the least significance difference (LSD) test; Significance at p-value (<0.001, <0.01, <0.05) have been given respective symbols in the tables.

**RESULT**

Table 1 represents the changes in the body weight in control and experimental groups of rats. A gradual gain in body weight was observed in the control group of rats whereas there was a significant (p<0.001) increase in body weight in group IV rats which was significant (p<0.001) higher than that of control group rats. Coadministration of chrysin to STZ induced rats led to significant gain in body weight in group IV rats compared to group II animals. However significant difference was observed between group II and group IV animals.

Table 2 demonstrates that the level of blood glucose in control and experimental animals. Control rats did not have any significant variation in the blood glucose throughout the experiment. In STZ induced diabetic rats there was a significant (p<0.001) and sustained raise in blood glucose level when compared with control animals. There was no significant change in the level of blood glucose in chrysin alone treated animals, it was found to be similar to those of control group of rats. Coadministration of chrysin to STZ induced rats observed no significant change in blood glucose level when compared with STZ induced diabetic rats. This suggested that chrysin prevented the development of hyperglycemia and it has anti-diabetic property.

**Table 1: Effect of chrysin on the body weight of control and experimental groups of rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>205±3.74</td>
<td>211±2.44</td>
<td>219±3.28</td>
<td>228±5.09</td>
<td>235±7.74</td>
<td>245±4.47</td>
</tr>
<tr>
<td>II</td>
<td>205±3.52</td>
<td>196±2.09</td>
<td>192±1.67</td>
<td>a***</td>
<td>a***</td>
<td>a*** 172±4</td>
</tr>
<tr>
<td>III</td>
<td>207±3.74</td>
<td>214±3.80</td>
<td>221±3.74</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IV</td>
<td>202±1.78</td>
<td>207±1.09</td>
<td>213±2.09</td>
<td>b***</td>
<td>b***</td>
<td>b***</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P values: ***<0.001, **<0.01, <0.05, ns-non-significant.

Table 2: Change in blood glucose levels in each experimental group of animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>85±4.4</td>
<td>90±2.52</td>
<td>86±1.7</td>
<td>89±2.09</td>
<td>86.6±1.03</td>
<td>88±2.19</td>
</tr>
<tr>
<td>II</td>
<td>90±1.26</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
</tr>
<tr>
<td>III</td>
<td>87±1.09</td>
<td>250±3.16</td>
<td>270±4.42</td>
<td>281.5±4.88</td>
<td>289±2.09</td>
<td>296±4.73</td>
</tr>
<tr>
<td>IV</td>
<td>90±2.82</td>
<td>93±1.54</td>
<td>89.66±1.36</td>
<td>88.66±2.06</td>
<td>90±1.78</td>
<td>85.6±3.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P value: ***<0.001, **<0.01, *<0.05, ns-non-significant.

Table 3. 4 represents the level of blood urea and serum creatinine of control and experimental group of rats. The levels of blood urea and serum creatinine in control and group III animals were found to be near normal throughout the study. There was significant (p<0.001) increase in the levels of blood urea and serum creatinine in STZ induced diabetic rat from fourth week onwards when compared with control animals. The observed reduced level of blood urea and serum creatinine in group IV animals might be due to coadministration of chrysin, suppressed the elevation of urea and creatinine, suggested the renoprotective action of chrysin.
The levels of glycosylated hemoglobin and plasma insulin in experimental groups of rats represented in Table 6. A significant (p<0.001) increase in the level of glycosylated hemoglobin and significant (p<0.001) decrease in the level of plasma insulin were found in STZ induced diabetic rats from twelveth week when compared with control rats. The observed significant (p<0.001) decrease in the level of glycosylated hemoglobin and significant (p<0.001) increase in the level of insulin in group IV animals when compared with group II animals might be due to chrysin coadministration. The effect was more distinct in the group of rats treated with chrysin alone.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>7.16±0.06</td>
<td>9.5±0.07</td>
<td>6.4±0.17</td>
<td>7.25±0.06</td>
</tr>
<tr>
<td>Plasma Insulin (μu/ml)</td>
<td>13.9±0.10</td>
<td>6.5±0.17</td>
<td>6.5±0.17</td>
<td>12.75±0.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

Table 5 indicates the level of serum protein in experimental animals. There was a significant (p<0.001) decrease in serum protein level in STZ induced diabetic animals from eighth week when compared to control group whereas the level of serum protein in group III and IV animals was similar to control animals. The observed level of serum protein in group IV animals might be due to chrysin coadministration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.60±0.014</td>
<td>0.58±0.034</td>
<td>0.53±0.019</td>
<td>0.50±0.026</td>
<td>0.50±0.014</td>
<td>0.48±0.019</td>
</tr>
<tr>
<td>II</td>
<td>0.88±0.05</td>
<td>a***</td>
<td>0.59±0.089</td>
<td>1.66±0.014</td>
<td>1.85±0.10</td>
<td>2.61±0.71</td>
</tr>
<tr>
<td>III</td>
<td>0.60±0.028</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IV</td>
<td>0.62±0.046</td>
<td>b***</td>
<td>0.57±0.033</td>
<td>0.55±0.034</td>
<td>0.50±0.026</td>
<td>0.46±0.041</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P value: ***<0.001, **<0.01, *<0.05, ns-non-significant.

Values are expressed as mean ± SD of six animals. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

Table 3: Effect of chrysin on the level of blood urea of control and experimental group of rats Blood Urea (mg/dl)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.75±0.27</td>
<td>7.95±0.33</td>
<td>7.97±0.35</td>
<td>8.10±0.22</td>
<td>8.15±0.30</td>
<td>8.23±0.22</td>
</tr>
<tr>
<td>II</td>
<td>7.62±0.41</td>
<td>a**</td>
<td>6.87±0.39</td>
<td>6.4±0.25</td>
<td>5.87±0.26</td>
<td>4.91±0.34</td>
</tr>
<tr>
<td>III</td>
<td>7.5±0.54</td>
<td>ns</td>
<td>7.90±0.54</td>
<td>8.125±0.51</td>
<td>8.20±0.51</td>
<td>8.36±0.48</td>
</tr>
<tr>
<td>IV</td>
<td>7.35±0.47</td>
<td>ns</td>
<td>7.79±0.60</td>
<td>7.82±0.55</td>
<td>7.99±0.51</td>
<td>8.125±0.51</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

The levels of glycosylated hemoglobin and plasma insulin in experimental groups of rats represented in Table 6. A significant (p<0.001) increase in the level of glycosylated hemoglobin and significant (p<0.001) decrease in the level of plasma insulin were found in STZ induced diabetic rats from twelveth week when compared with control rats. The observed significant (p<0.001) decrease in the level of glycosylated hemoglobin and significant (p<0.001) increase in the level of insulin in group IV animals when compared with group II animals might be due to chrysin. The effect was more distinct in the group of rats treated with chrysin alone.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>7.16±0.06</td>
<td>9.5±0.07</td>
<td>6.4±0.17</td>
<td>7.25±0.06</td>
</tr>
<tr>
<td>Plasma Insulin (μu/ml)</td>
<td>13.9±0.10</td>
<td>a***</td>
<td>ns</td>
<td>b***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals from each group. Comparison between a-Group I and Group II, b-Group II and Group IV, c-Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.
The level of urinary glucose in experimental animals was indicated in Table 7. A significant (p<0.001) excretion of glucose in urine was found in STZ induced diabetic rats from fourth week onwards whereas there was no glucose in the urine of group I, III and IV animals.

Table 8, 9 represents the level of urinary urea and creatinine in experimental rats. There was a significant (p<0.001) increase in the level of urinary urea and significantly (p<0.001) reduced level of creatinine were found in group II animals from eighth week when compared with control animals. The level of urea and creatinine in urine of group III and IV animals were found to be as similar to control group of rats.

The levels of excretion of protein in urine of experimental animals were represented in Table 10. Excretion of protein in urine was not observed in any rat from group I, III and IV. However, there was a significant (p<0.001) and sustained increase in urinary protein after eight weeks in STZ induced diabetic rats.

**Table 7: Effect of chrysin on the level of urinary glucose of control and experimental group of rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>II</td>
<td>Nil</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
</tr>
<tr>
<td></td>
<td>0.5±0.06</td>
<td>1.0±0.14</td>
<td>1.45±0.13</td>
<td>2±0.14</td>
<td>2±0.2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>IV</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a - Group I and Group II. P value: ***<0.001

**Table 8: Effect of chrysin on the level of urinary urea of control and experimental group of rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>37.8±0.44</td>
<td>38.5±0.56</td>
<td>38.5±0.58</td>
<td>37±0.40</td>
<td>38.25±0.82</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>36.5±0.42</td>
<td>ns</td>
<td>38.8±0.52</td>
<td>a***</td>
<td>74±0.10</td>
<td>96±0.89</td>
</tr>
<tr>
<td>III</td>
<td>36.5±1.04</td>
<td>ns</td>
<td>37.5±1.08</td>
<td>a***</td>
<td>48±0.83</td>
<td>38±1.98</td>
</tr>
<tr>
<td>IV</td>
<td>37.4±0.74</td>
<td>b***</td>
<td>37.7±0.81</td>
<td>39±0.83</td>
<td>39.6±0.83</td>
<td>39.2±0.56</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a - Group I and Group II, b - Group II and Group IV, c - Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

**Table 9: Effect of chrysin on the level of urinary creatinine of control and experimental group of rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>53.1±1.41</td>
<td>53.3±1.09</td>
<td>55.1±2</td>
<td>60.2±2.09</td>
<td>60±2.36</td>
<td>65.1±3.16</td>
</tr>
<tr>
<td>II</td>
<td>52.5±0.77</td>
<td>ns</td>
<td>51.2±1.01</td>
<td>a***</td>
<td>50±1.13</td>
<td>50±2.82</td>
</tr>
<tr>
<td>III</td>
<td>53.2±1.41</td>
<td>ns</td>
<td>53.4±2.09</td>
<td>c*</td>
<td>63.2±1.41</td>
<td>63±1.78</td>
</tr>
<tr>
<td>IV</td>
<td>55±2.82</td>
<td>b***</td>
<td>57.1±4</td>
<td>60±3.34</td>
<td>65.1±2.60</td>
<td>65.2±2.28</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a - Group I and Group II, b - Group II and Group IV, c - Group I and Group III

P values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

**Table 10: Effect of chrysin on the level of urinary protein of control and experimental group of rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>II</td>
<td>Nil</td>
<td>0.7±0.04</td>
<td>1.46±0.10</td>
<td>2±0.14</td>
<td>2.5±0.14</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>IV</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD of six animals from each group. Comparison between a - Group I and Group II, p value: ***<0.001

Table 11 indicates the level of creatinine clearance in experimental animals. Creatinine clearance was taken as a parameter to assess GFR. In the early weeks of diabetes there was a normal creatinine clearance and in the later weeks there was a gradual decline in GFR in group II animals. The GFR was found normal in early weeks in group III and IV animals and decreased rise in later weeks when compared to control animals and there was significant (p<0.001) rise GFR in later weeks when compared to group II animals.


Table 1: Effect of chrysin on the level of creatinine clearance of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 week</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>16 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.19±0.017</td>
<td>1.20±0.019</td>
<td>1.31±0.018</td>
<td>1.46±0.01</td>
<td>1.41±0.01</td>
<td>1.53±0.017</td>
</tr>
<tr>
<td>II</td>
<td>1.21±0.02</td>
<td>a***</td>
<td>a***</td>
<td>1.22±0.025</td>
<td>a***</td>
<td>a***</td>
</tr>
<tr>
<td>III</td>
<td>1.18±0.026</td>
<td>c***</td>
<td>c***</td>
<td>1.37±0.02</td>
<td>1.50±0.014</td>
<td>1.57±0.04</td>
</tr>
<tr>
<td>IV</td>
<td>1.21±0.018</td>
<td>1.18±0.03</td>
<td>1.23±0.014</td>
<td>1.46±0.014</td>
<td>1.44±0.014</td>
<td>1.49±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as mean SD of six animals from each group. Comparison between a- Group I and Group II, b- Group II and Group IV, c- Group I and Group III.

P value: ***<0.001, **<0.01, *<0.05, ns- non-significant.

**DISCUSSION**

Diabetic nephropathy is a leading cause of end-stage renal failure, accounting for 35-40% of all new cases requiring dialysis therapy worldwide. Early diabetic nephropathy is characterized by hyperfiltration, microalbuminuria, renal and glomerular hypertrophy, mesangial matrix accumulation and thickening of the glomerular basement membrane. In the later stages, when diabetic nephropathy progresses, patients develop proteinuria and their glomerular filtration rate decline eventually leading to end-stage renal disease. Hyperglycemia, hyperlipidemia, hypertension and also proteinuria itself, contribute to progression of renal damage.

Results of the study confirm that STZ, commonly used diabetogenic agent in experimental animals, causes hyperglycemia, polyuria, macroproteinuria as well as decrease in GFR. Under such conditions hyperglycemia is due to the damage of the beta-cells.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to catabolism of structural proteins leading to significant reduction in the body weight gain of diabetic nephropathy rats, which was observed in the present study. The reduction in the body weight of diabetic nephropathy rats might have occurred as a result of catabolism of structural proteins due to scarcity of carbohydrate as energy source. Weight loss during diabetes is mainly related to urinary glucose excretion because cells are unable to utilize glucose. Another factor is the osmotic diuresis resulting in hyperosmotic dehydration. A significant increase in the body weight was observed in STZ induced rats administered with chrysin which could be due to the protective effect of chrysin in controlling muscle wasting and also due to the improvement in insulin secretion from the pancreatic beta cells and glycemic control. The fundamental mechanism underlying hyperglycemia involves over production of glucose by excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization by the tissues. Persistent hyperglycemia, is a factor in the development and progression of the complications of diabetes mellitus. Reports have shown that the level of the blood glucose was elevated in STZ induced diabetic rats. In the present study, we have also observed a marked elevation in blood glucose level of STZ induced rats and there was no rise in the level of blood sugar in chrysin coadministered STZ induced rats. This data suggested that coadministration of chrysin with STZ prevents the development of diabetic nephropathy by maintaining blood glucose level to normal suggesting insulin secretory effect and antihyperglycemic activity of chrysin.

Glycosylated hemoglobin remains the gold standard biochemical marker for the assessment of diabetes. A high glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. This condition favours reduction in the level of total hemoglobin and elevation in glycosylated hemoglobin, which is directly proportional to blood glucose. The observed high levels of glycated hemoglobin in STZ induced rats after twelve weeks reveals poor glycemic control. Coadministration of chrysin to STZ-induced rats reduced the formation of glycosylated hemoglobin by virtue of its normoglycemic activity. Since the glycosylation of protein is an oxidation reaction, flavonoids should be able to prevent the real targets they are, namely non-enzymatic glycation of proteins in animals.

In the present study, we have observed a significant decrease in the levels of insulin in STZ-induced diabetic rats. Insulin deficiency is manifested in a number of biochemical and physiological alterations. The simultaneous administration of chrysin and STZ prevented the deficiency of insulin and enhanced the insulin secretion which suggested the insulin secretory effect of chrysin.

Urine glucose estimation study revealed that animals administered with chrysin and STZ prevented the excretion of glucose in urine, whereas there was significant increase in the level of glucose in urine of STZ induced diabetic rats from the fourth week onwards. The observed normal level of blood glucose and total absence of urinary glucose in rats administered with chrysin alone suggested antidiabetic activity of chrysin.

The diabetic hyperglycemia induces the elevation of the blood urea and serum creatinine in diabetic rats, which are considered as significant markers of renal dysfunction. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea in blood. Increased plasma creatinine level and BUN are indication of the development of diabetic nephropathy in rats. In the present investigation there was a significant elevation in the levels of blood urea and serum creatinine from the fourth week of the study in STZ induced diabetic rats. Our study revealed that coadministration of chrysin with STZ to rats prevented the development of diabetic nephropathy by lowering blood urea and serum creatinine. This could be explained that there was increased clearance of blood urea and creatinine by the kidney or that there was decreased protein degradation.

The observed increased excretion of urinary urea and decreased excretion of creatinine indicates the development of diabetic nephropathy in STZ induced rats. Whereas the rats coadministered with chrysin and STZ demonstrated reduced level of urinary urea and increased level of urinary creatinine. We also observed normal level of urea and creatinine in urine of rats administered with chrysin alone. This report suggested that chrysin prevented the progression of diabetic nephropathy and protected the kidney from further damage.

Serum creatinine concentration is widely interpreted as a measure of the GFR and is used as an index of renal function in clinical practice. The end-stage of diabetic renal disease is usually characterized by changes in both proteinuria and subsequent decline in GFR. Development of lesions in the glomerular capillaries of the kidneys allows protein to escape because of changes in the basement membrane.

CONCLUSION

The results of the present study demonstrates that reduced level of protein in serum, GFR and development of proteinuria in STZ induced rats, clearly suggested that the development of diabetic
nephropathy and coadministration of chrysin attenuates the development of proteinuria and elevated the creatinine clearance level and there by maintains GFR to normal. This suggested that chrysin has antidiabetic and antidiabetic nephropathy effect. Further studies with the compound will help in designing pharmacological active compound that can be administered along with insulin in diabetic mellitus patients or administered in early diabetic nephropathy patients that will quench the secondary complications of diabetic mellitus.

REFERENCES


Effect of chrysin on nephroprotective and antioxidant status in streptozotocin induced diabetic nephropathy rats

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ABSTRACT
Prevention or reversal of diabetic nephropathy is a major challenge in the current management of diabetes. Oxidative stress and changes in antioxidant activity are considered to play a key role in the pathogenesis of diabetic nephropathy. The aim of the present study is to evaluate whether administration of chrysin could prevent the progression of diabetic nephropathy induced by oxidative stress in rats. Wistar male albino rats were allocated into four groups. Group I rats received DMSO, Group II rats received STZ, Group III rats received chrysin and Group IV rats received STZ and chrysin. Blood samples were collected for the estimation of blood glucose, urea, uric acid and creatinine. Kidney of the sacrificed rats were excised and homogenized for the assay of thiobarbituric acid reactive substances (TBARS), non-enzymatic antioxidants (glutathione, vitamin C, vitamin E and vitamin A) and enzymatic antioxidants Superoxide Dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and glutathione transferase (GST). There was a significant (p<0.001) increase in the level of blood glucose, urea, uric acid, creatinine and lipid peroxidation product TBARS in STZ induced diabetic group and significant (p<0.001) decrease in chrysin treated group while the levels of glutathione, vitamin C, vitamin E, vitamin A, activities of SOD, CAT, GPx and GST were significantly (p<0.001) reduced in STZ induced diabetic group and significant (p<0.001) increase in chrysin treated group. The findings of the present study concluded that chrysin treatment has beneficial effect on renal tissues subjected to STZ-induced oxidative stress by directly quenching lipid peroxides and indirectly enhancing production of endogenous antioxidants.

KEY WORDS: Chrysin, streptozotocin, diabetic nephropathy, antioxidant, lipid peroxidation, oxidative stress.

INTRODUCTION
Diabetes mellitus is a multifactorial disease characterized by hyperglycemia together with biochemical alterations of metabolism. These traits are hypothesized to be responsible for the damage to cell membrane, which in turn results in an elevated production of reactive oxygen species (ROS). The elevated generation of ROS and the simultaneous decline in antioxidative defense mechanisms observed in the diabetic patients could promote the development of late complications. It has been postulated that etiology of the complications of diabetes involve oxidative stress which result in cellular damage. Accumulating research suggest that oxidative stress play a key role in the pathogenesis of diabetic nephropathy. Diabetic nephropathy is a leading cause of end stage renal failure, accounting for 35 to 40% of all new case that require dialysis therapy World Wide. Diabetic nephropathy is characterized by persistent albuminuria, a decline in Glomerular filtration rate and arterial blood pressure. Despite current treatments involving glycemia and blood pressure control, many diabetic patients are still prone to developing kidney failure. There is a continuous search for agents that could prevent the production of reactive oxygen species. Plant kingdom is found to possess certain non-toxic chemicals which act as antioxidants and can scavenge the free radicals. Flavonoids are natural antioxidants that exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, antiallergic, antithrombotic and vasodilatory action. Flavonoids can exert their antioxidant activity by various mechanisms, by scavenging or quenching free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.

Chrysin is a natural and biological active flavones extracted from species like passiflora, pelargonium and pinaceae. Like other flavonoids, chrysin exhibits many biological activities and pharmacological effects including antioxidant, antiinflamatory, antiancer and antidiaibetogenic. Chrysin involves in the enchancement of endogenous antioxidant enzymes and decreases the total lipid peroxide. Chrysin was able to prevent the oxidative damage induced by ccl4 in liver, brain, kidney and hemolyase of male wistar rats. Chrysin also offers protection against free radical-mediated stress in rats with ethanol induced liver injury. Sufficient work has not been done to study its nephroprotective activity. The present study was designed to carry out systemic investigation of the protective effect of chrysin on diabetic nephropathy in rats induced by STZ.

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MATERIALS AND METHODS

Animals

Male albino rats of wistar strain with a body weight ranging from 180 to 200g were procured from Saveetha university, Chennai, India and were maintained in an air-conditioned room (25±3°C) with a 12h light/12h dark cycle. Feed and water were provided ad libitum to all the animals. The study was approved by the Institutional Animal Ethical Committee of Saveetha University, Chennai, IAEC. No. Biochem BWC/009/2010.

Chemicals

Streptozotocin and Chrysin were purchased from sigma chemicals co (St. Louis, Mo, USA). All other chemicals used in this study were of analytical grade obtained from SRL chemicals, Mumbai, India.

Experimental induction of diabetic mellitus

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (50mg/kg body weight) in 0.1M citrate buffer, pH 4.5 in a volume of 0.1ml per rat, after 16h fasting. Seventy two hours after STZ administration, the blood glucose level of each rat was determined for confirmation of diabetes. Rats with blood glucose level above 250mg/dl were considered for further study.

Experimental design

The animals were randomly divided into four groups of six animals each as given below

**Group I:** Rats received Dimethyl Sulphoxide (1% DMSO) as vehicle i.p for 20 weeks and referred as positive control rats.  
**Group II:** Rats were administered i.p a single dose of STZ 50mg/kg body weight and served as diabetic rats.  
**Group III:** Rats were treated with chrysin 20mg/kg body weight dissolved in 1% DMSO i.p for 20 weeks to assess the toxicity if any induced by chrysin and rats were referred as drug control.  
**Group IV:** Rats were administered with STZ 50mg (as in group II) along with chrysin 20mg (as in group III) and were referred as treated rats.

Sample collection

The blood was collected from orbital plexus by prickling a needle under ketamine anesthesia for the estimation of glucose, urea, uric acid and creatinine.

Biochemical Parameters

Blood glucose was estimated by o-Toluidine method, urea was measured by Diacetyl Monooxime method, uric acid was quantified by Phospho tungstate method and serum creatinine measured by alkaline picrate method.

At the end of the experimental period (20 weeks), the animals from each experimental group were starved for 16h and sacrificed by ketamine injection. Kidneys were removed immediately, washed with ice-cold physiological saline to remove the blood. The tissues were sliced and homogenized in ice-cold 0.1M Tris buffer (pH 7.4). The homogenates were centrifuged at 48X in a cold centrifuge. The supernatants were collected and used for the assessment of thiobarbituric acid reactive substances, Glutathione, Vitamin C, Vitamin E and Vitamin A by the method of Hunter et al, Moron et al, Omaye et al, Baker et al and Bessey et al respectively. Super oxide dismutase, Catalase, Glutathione peroxidase and Glutathione transferase were estimated by the method of Misra and Fridrich, Sinha, Rotruck et al and Habig et al respectively.

Statistical analysis

The values are expressed as mean ±SD for six animals in each group. Differences between the groups were assessed by one-way analysis of variance (ANOVA) using SPSS software package for windows. Post hoc testing performed for inter-group comparisons using the least significance difference (LSD) test. Significance at p-value (<0.001, <0.01, <0.05) have been given respective symbols in the tables.

RESULT

Table 1 represents the effect of chrysin on the levels of blood glucose, urea, serum uric acid and creatinine of experimental groups of rats. The levels of blood glucose, urea, serum uric acid and creatinine were found to be significantly (p<0.001) increased in STZ induced diabetic nephropathy rats when compared with the control rats. The levels of blood glucose, urea, serum uric acid and creatinine in group III were found to be near normal. The significant (p<0.001) decrease in the level of blood glucose, urea, serum uric acid and creatinine observed in group IV might be due to coadministration of chrysin, suppressed the elevation of glucose, urea, uric acid and creatinine, suggested the nephroprotective action of chrysin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>88±2.19</td>
<td>296±4.73</td>
<td>85.5±3.01</td>
<td>91.6±1.50</td>
</tr>
<tr>
<td>Urea</td>
<td>20±1.26</td>
<td>68±0.63</td>
<td>19±0.89</td>
<td>23±2.09</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>2.87±0.13</td>
<td>5±0.22</td>
<td>2.87±0.23</td>
<td>4.20±0.18</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.48±0.019</td>
<td>2.16±0.071</td>
<td>0.46±0.041</td>
<td>0.50±0.014</td>
</tr>
</tbody>
</table>

Values are expressed as **mean ± SD** of six animals from each group.  
Comparison between a- Group I and Group II, b- Group II and Group IV, c- Group I and Group III. P values: ***<0.001, **<0.01, *<0.05, ns- non-significant.

Figure I represent the effect of chrysin on the levels of glutathione, vitamin C, vitamin E and vitamin A in kidneys of experimental groups of animals. There was a significant (p<0.001) decrease in the levels of glutathione, vitamin C, vitamin E and vitamin A in the STZ induced diabetic nephropathy rats when compared with the control rats. The levels of glutathione, vitamin C, vitamin E and vitamin A in group III rats were found to be near normal. The significant (p<0.001) increase in the levels of glutathione, vitamin C, vitamin E and vitamin A in group IV animals when compared with group II animals might be due to the administration of chrysin. These data indicates that the protective effect of chrysin against renal damage.
Figure I: Effect of chrysin on the levels of glutathione, vitamin C, vitamin E and vitamin A in kidneys of experimental groups of animals

Values are expressed as mean ± SD of six animal from each group. Comparison between a- Group I and Group II, b- Group II and Group IV and c- Group I and Group III

p Values: ***<0.001, **<0.01, *<0.05, ns- non significant

Units for Glutathione in µmole/min/mg of protein, vitamin C, vitamin E and vitamin A in µg/mg of protein

Table 2. Effect of chrysin on the level of lipid peroxidation products as TBARS and the activities of antioxidants enzymes SOD and Catalase in the kidneys of experimental groups of animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>266.66±25.8</td>
<td>625±27.38</td>
<td>241.33±20.4</td>
<td>275±27.38</td>
</tr>
<tr>
<td>SOD</td>
<td>15.75±0.97</td>
<td>8.08±0.09</td>
<td>16.50±1.65</td>
<td>14.55±1.22</td>
</tr>
<tr>
<td>Catalase</td>
<td>69.44±4.30</td>
<td>39.16±0.91</td>
<td>70.83±4.56</td>
<td>58.05±6.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals from each group. Comparison between a- Group I and Group II, b- Group II and Group IV, c- Group I and Group III

p Values ***<0.001, **<0.01, *<0.05, ns- non significant

Units - Lipid peroxidation-nmoles of malonaldehyde/mg of protein, SOD-Units/mg of protein and Catalase- ?moles of hydrogen peroxide/mg of protein

DISCUSSION

Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes. Free radicals are formed disproportionately during diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of protein. The hyperphysiological burden of free radicals can cause an imbalance in the homeostatic phenomena between oxidants and antioxidants in the body and this imbalance can lead to oxidative stress. Hyperglycemia promotes the liberation of oxygen free radicals, which reduces the antioxidant potential of the body and has been shown to damage pancreatic beta- cells. Antioxidants can scavenge free radicals against damage and decay and they have an important role in biological systems, there- by providing a way to prevent or treat diabetic mellitus. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetic mellitus. Reduced oxidative stress in the diabetic condition has been observed in experimental animals after the administration of certain polyphenols.
Persistent hyperglycemia, a factor in the development and progression of the complications of diabetes mellitus\(^3\). Reports have shown that the level of blood glucose was elevated in STZ induced diabetic rats. In the present study, we have also observed a marked elevation in blood glucose level of STZ-induced rats and there was no rise in the level of blood sugar in chrysin coadministered STZ induced rats. Chrysin by its ability to scavenge free radicals and to exhibit lipid peroxidation prevents STZ induced oxidative stress and protects beta-cell and decrease blood glucose level. This data suggested that coadministration of chrysin with STZ prevents the development of diabetic nephropathy by maintaining the blood glucose levels to normal suggesting insulin secretory effect and anti hyperglycemic activity of chrysin. Impaired balance of nitrogenous coupled with lowered protein synthesis leads to increased concentration of urea in blood. The body cannot efficiently put the nitrogenous waste into gaseous form and exhale it. Urea, uric acid and creatinine which are the major nitrogenous waste products are normally low in the blood since it is excreted in the urine continuously. When the kidney fails to filter out this nitrogenous waste due to increased blood glucose level or due to kidney failure and the nitrogenous wastes get accumulated in the blood and thus can lead to unconsciousness and death. The diabetic hyperglycemia induces the elevation of the blood urea, uric acid and creatinine in diabetic rats, which are considered as significant markers of renal dysfunction.

In hyperglycemic condition generates oxidative stress in reactive oxygen species which in turn cause lipid peroxidation and membrane damage\(^3\). Lipid peroxidation is a free-radical mediated propagation of oxidative insult to polyunsaturated fatty acids involving several types of free radicals and termination occurs through enzymatic means or by free radical scavenging by antioxidants\(^3\). Measurement of plasma TBARS is considered as the most reliable marker to assess the extent of tissue damage in pathological conditions\(^3\). In this study there was a significant elevated level of TBARS in kidney of diabetic rats which was recovered by coadministration of chrysin this can be due to the ability of the chrysin to decrease the lipid peroxidation and there by activating the antioxidants to scavenge the free radical responsible for the lipid peroxidation during diabetes. The increased production of free radicals may also lead to the depletion of protective physiological moieties such as glutathione, vitamin C, vitamin E and vitamin A in diabetic rats. Ascorbate has received much attention as a reducing agent since its discovery, and it has been recognized as an outstanding plasma antioxidant\(^3\). In the present experiment, concentration of vitamin C in kidneys of diabetic rats were shown to decrease significantly. This result is in accordance with\(^3\). The decrease in vitamin C may be ascribed to its enchanced utilization due to increased oxidative stress caused by diabetes. Coadministration of chrysin to diabetic nephropathy rats increased the level of tissue vitamin C.

Vitamin E is a major lipid soluble chain breaking antioxidant. In the present experiment, vitamin E levels in kidney of diabetic rats were decreased significantly. In this context\(^28\) also reported increased level of vitamin E in plasma. This can be due to increased peroxidation which was recovered after the treatment with chrysin.

Vitamin A acts as a powerful free radical scavenger, for singlet oxygen, and as a chain breaking antioxidant\(^4\). The function of vitamin A as a radical scavenging antioxidant can provide protection from oxidative damage in cells\(^4\). Vitamin A likely contribute to the inhibition of lipid peroxidation by promoting the recycling of vitamin E\(^4\). Decreased level of vitamin A in the kidney of diabetic nephropathy rats may be due to an increased level of oxygen radical in tissues of STZ induced diabetic animals. Administration of chrysin rescued the depressed levels back to normal levels. This effect could be attributed to the regeneration of vitamin A from its radical.

Glutathione is an important intracellular peptide, that exhibit multiple functions ranging from antioxidant defense to modulation of cell proliferation\(^4\). It has been suggested that the hyperglycemia leads to enhanced activity of the polyl pathway to promote GSH depletion, which creates a redox imbalance in diabetic hepatocytes, nephrons and pancreatic cells. This state can lead to a depressed defense against oxidative stress. Sustained oxidative insult cause lipid peroxidation in cellular membrane and leads to accumulation of malondialdehyde, which is a stable end product of lipid peroxidation. These alterations have been implicated in the development of long term complications associated with diabetes\(^3\). Decreased oxidative stress in diabetic nephropathy rats administered with chrysin could result in the restoration of glutathione levels in the plasma. Glutathione through its significant reducing power contributes to the recycling of other antioxidants such as vitamin C and vitamin E, that have become oxidized\(^4\).

Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of the free radicals. SOD protects tissue against oxygen free radicals by catalyzing the removal of superoxide radical, converting it into hydrogen peroxide and molecular oxygen, which both damage the cell membrane and other biological structures\(^4\). Catalase is a heme protein, which catalyzed the reduction of hydrogen peroxides and protected the tissues from highly reaction hydroxyl radicals. The decreased activity of SOD and Catalase in the kidney observed in this study may result in a number of deleterious effects caused by the accumulation of superoxide radicals and hydrogen peroxide. Administration of chrysin increased the activity of SOD and catalase in diabetic nephropathy rats, there by indicating that chrysin exhibited free radical scavenging activity, which could rescue pathological alterations caused by the presence of superoxide radicals. This effect may involve mechanisms related to scavenging activity.

Glutathione peroxidase, an enzyme with selenium and Glutathione transferase catalyze the reduction of hydrogen peroxide and hydroperoxides to non toxic products. Reduced activities of GPx and GST were observed in our study. Many researchers also reported a decrease in the activities of these antioxidant enzymes in the liver and
kidneys of diabetic rats. The reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme. The activity of these enzymes were restored in chrysin administered diabetic nephropathy rats and this result reflect the antioxidant potency of the chrysin, which by reducing blood glucose levels, prevent glycation and improve the activity of enzymatic and non-enzymatic antioxidants.

CONCLUSION
The present findings suggest that the chrysin is non-toxic, since no marked changes were observed in the normal rats fed with the chrysin. Thus chrysin was considered to be safe for long-term treatment in diabetic condition. The chrysin showed potent antidiabetic activity apart from this, the chrysin also improved the activity of enzymatic and non-enzymatic antioxidants, there by scavenging the free radical that initiates the lipid peroxidation. The decreased level of urea, uric acid and creatinine in the treated rats clearly indicates that the chrysin protects the diabetic rats from STZ induced renal damage.

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REFERENCES
20. Jaffe M. Concerning the precipitate produced in normal urine by picric acid and a new reaction of creatinine. Physio Chem. 1886; 10: 91-400
22. Moron MS, Defiere JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. J Biochem Biophys Acta 1979; 582: 67-68
23. Omaye ST, Turnbull JD, Sauberlich HE. Selected method for the determination of ascorbic acid in animal cells, tissues and fluid. In: Meth in Enzymol 1979; 62: 3-11
24. Baker H, Frank O, De Angelis, Freingold S. Plasma toco-pherol in man at various times after ingesting free or acety-
44. Lu SC. Regulation of hepatic glutathione synthesis, current concepts and controversies. FASEB J 1999; 13: 1169-1183
48. Hodgin EK and Fridovich I. Biochemistry 1975; 24: 5294-5299

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