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/kgbw), (Group-III) rats received chrysin (20mg/kgbw) and (Group-IV) rats received STZ (50mg/kgbw and chrysin 20mg/kgbw). 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 was determined. The levels of blood glucose, urea, serum creatinine total urinary protein, urine urea, creatinine, protein and glomerular filtration rate was significantly (p<0.001) increased and Glomerular filtration rate was significantly (p<0.001) reduced in diabetic nephropathy rats. Coadministration of chrysin to STZ induced 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 extra cellular 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 afore mentioned 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 pharmacological activities such as anti-inflammatory, antioxidant, antihypertensive, antidiabetogenic and anticancer. Chrysin also has the potency for clinical and 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 acclimatised 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 pricking 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.pfor 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 111: 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)⁴. 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 method⁶, serum and urinary creatinine were measured by alkaline picrate method⁵. Urinary protein was quantified by Lowry's method²⁷. Glycosylated Hemoglobin was determined by the method of Nakay 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) decrease in STZ induced diabetic nephropathy rats when compared with control rats. Administration of chrysin alone in group III rats has significant (p<0.001) gain in body weight when compared with group II animals. Co-administration of chrysin with STZ induced rats led to significant gain in body weight in group IV rats compared to group II rats. However significant difference was observed between group II and group IV animals.

Table 2 demonstrates 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. Co-administration 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 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 co-administration of chrysin, suppressed the elevation of urea and creatinine, suggested the renoprotective action of chrysin.
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 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.

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 twelfth 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.
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>Urinary glucose (g/dl)</th>
<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>
<td>Nil</td>
</tr>
<tr>
<td>II</td>
<td>Nil</td>
<td>a***</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>
</tr>
<tr>
<td>III</td>
<td>Nil</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>
<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 values: ***<0.001, **<0.01, *<0.05, ns-non-significant.

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

<table>
<thead>
<tr>
<th>Urinary urea (mg/dl)</th>
<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±0.44</td>
<td>38.1±0.56</td>
<td>38.5±0.58</td>
<td>37.6±0.40</td>
<td>38.2±0.2</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>36.5±0.42</td>
<td>ns</td>
<td>a***</td>
<td>56.5±0.57</td>
<td>68±0.44</td>
<td>74.5±0.10</td>
<td>96±0.89</td>
</tr>
<tr>
<td>III</td>
<td>36.5±1.04</td>
<td>ns</td>
<td>c</td>
<td>37.8±1.14</td>
<td>37.2±1</td>
<td>38.4±0.83</td>
<td>38.1±0.98</td>
</tr>
<tr>
<td>IV</td>
<td>37.4±0.74</td>
<td>ns</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

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

<table>
<thead>
<tr>
<th>Urinary creatinine (mg/dl)</th>
<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>
<td></td>
</tr>
<tr>
<td>II</td>
<td>52.5±0.77</td>
<td>ns</td>
<td>a***</td>
<td>51.2±1.01</td>
<td>50.4±2.82</td>
<td>50.2±1.13</td>
<td>50±2.82</td>
</tr>
<tr>
<td>III</td>
<td>53.2±1.41</td>
<td>ns</td>
<td>c*</td>
<td>53.4±2.09</td>
<td>56.1±1.41</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>b***</td>
<td>57.1±4</td>
<td>60.3±3.34</td>
<td>65.1±2.60</td>
<td>65±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

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

<table>
<thead>
<tr>
<th>Urinary Protein (g/L)</th>
<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>
<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>
<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>
<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>
<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.
Filtration, microalbuminuria, renal and glomerular hypertrophy, accounting for 35-40% of all new cases requiring dialysis therapy worldwide. Early diabetic nephropathy is characterized by hyperglycemia due to the damage of the beta-cells.

Results of the study confirm that STZ, commonly used diabetogenic agent in experimental animals³, causes hyperglycemia, polyuria, macroproteinuria as well as decrease in GFR. Under such conditions glycogenolysis and gluconeogenesis and decreased utilization by the tissues⁴. Persistent hyperglycemia, is a factor in the development of diabetic nephropathy by maintaining blood glucose level to normal virtue of its normoglycemic activity. Since the glycosylation of protein is an oxidation reaction, flavonoids should be able to prevent the real pathogenic effects. Several researches have demonstrated that flavonoids attenuate hyperglycemia and there is reduced non-enzymatic glycation of proteins in animals⁶. 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.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting³¹ and increased level of urinary creatinine. We also observed normal loss of body weight, which is due to increased muscle wasting³¹ and protein turnover and may also due to the improvement in insulin secretion from the pancreatic beta cells and glycemic control. The observed increased excretion of urinary urea and decreased concentration of urea in blood⁴¹, Increased plasma creatinine level and BUN are indication of the development of diabetic nephropathy and protected the kidney from further damage. The observed increased excretion of urinary urea and decreased creatinine concentration of urine in rats administered with chrysin alone suggested antidiabetic activity of chrysin.

Table 11: 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>1.33±0.017</td>
</tr>
<tr>
<td>II</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>a***</td>
<td>0.68±0.032</td>
<td>0.63±0.02</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>1.10±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. The observed increased excretion of urinary urea and decreased concentration of urea in blood⁴¹, Increased plasma creatinine level and BUN are indication of the development of diabetic nephropathy and protected the kidney from further damage. The observed increased excretion of urinary urea and decreased creatinine concentration of urine in rats administered with chrysin alone suggested antidiabetic activity 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 makers of renal dysfunction. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea in blood⁴⁴. 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 where 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 serum 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 is reduced by the administration of chrysin.
nephropathy and coadministration of chrysin attenuate 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


