Chapter # 6: NEPHROPROTECTIVE EFFECTS OF NARINGENIN ON STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY IN RATS

Diabetic nephropathy is one of the most serious complications in diabetes mellitus, and can cause end-stage renal disease [184]. DN also known as diabetic glomerulosclerosis or diabetic kidney disease is a frequently occurring and dangerous complication of diabetes mellitus [185]. The most patients with ESRD have to receive renal replacement therapy through either dialysis or kidney transplantation due to the diabetic renal failure in the end. With the changes of working and living styles, as well as the unhealthy dietary habits, the DN incidences have rapidly increased worldwide in the past few decades and become a serious health concern to both individual and public [186,187].

Hyperglycemia is a critical factor in the development of DN and is associated with an increase in matrix production and basement membrane thickening. Hence, glycemic control could slow the development of diabetic nephropathy [188]. High intracellular glucose concentration induces increased flux through the polyol pathway, enhanced formation of advanced glycation end products (AGEs), activation of PKC[42] and increased shunting of excess glucose through the hexosamine pathway, collectively leading to the overproduction of ROS, which are centrally involved in the pathogenesis [63]. Sustained hyperglycemia leads to the production of AGEs through nonenzymatic binding between proteins and sugars [46]. AGEs accumulate in the tissues and contribute to the development of microvascular complications of DM [47]. Diabetic nephropathy which is characterized by increased matrix proteins leading to decreased GFR is considered as a marker for the progression of the disease. Elevation of serum creatinine and BUN in diabetic rats is used as an index of altered
GFR in diabetic nephropathy. Blood creatinine is directly associated with GFR and creatinine secretion rate and it tends to increase, relative to the extent of fibrosis in renal cortex mesangium.

Thickening of GBM is a characteristic early change in DN and it strongly correlates with urinary albumin excretion. GBM thickening is then followed by glomerular mesangial expansion which is a hallmark of DN and eventually leads to decline in renal function. Studies have shown mesangial expansion to be associated with both increased production and reduced degradation of ECM proteins, for example, type IV and I collagen, fibronectin and laminin [189]

Traditional Indian and Chinese medicine are being increasingly recognized worldwide. A series of bioactive compounds have been isolated from the medicinal herbs. Citrus fruits are considered to have renal protective activity. After thorough analysis found that naringenin was the major active ingredient in the citrus fruits. However, no reports have recorded the precise biological action of naringenin against diabetic nephropathy in rats. Therefore, the present study was designed with an aim to determine the protective effects of naringenin on diabetic nephropathy in rats.

6.1 RESULTS
6.1.1 Effects on body weight and kidney weight

Table 6.1 and figure 6.1 shows the effects of NARN on physical parameters of control and experimental animals. The body weights of rats administered with STZ were reduced significantly in comparison to the normal control group. The decreased body weight of STZ induced DN rats were increased in group III and group IV (positive control) compared to normal group. Rats treated with NARN alone showed
no significant difference in body weight status compared to control animals. But the kidney weights of DN rats were significantly increased compared with normal controls. However, administration of NARN (group III) and AG (group IV) were revert the weight of kidney to near normal range. Animals treated with NARN (group V) alone showed no significant difference in kidney weight as compared to control animals. The altered kidney to body weight index of group II animals were normalized in NARN treated groups.
Table 6.1: Effect of NARN on physical parameters in STZ-induced diabetic nephropathy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II DN</th>
<th>Group III DN + NARN</th>
<th>Group IV DN + AG (Positive control)</th>
<th>Group V NARN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight(g)</td>
<td>230.09±13.78^a</td>
<td>197.34±11.55^b</td>
<td>228.04±13.66^a</td>
<td>226.38±13.56^a</td>
<td>229.18±13.73^a</td>
</tr>
<tr>
<td>Kidney weight(g)</td>
<td>0.947±0.065^a</td>
<td>1.283±0.028^b</td>
<td>0.975±0.060^a</td>
<td>0.977±0.052^a</td>
<td>0.958±0.041^a</td>
</tr>
<tr>
<td>Ratio of the kidney weight to the body weight (%)</td>
<td>0.412±0.016^a</td>
<td>0.661±0.024^b</td>
<td>0.428±0.019^a</td>
<td>0.437±0.004^a</td>
<td>0.419±0.014^a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
DN- Diabetic Nephropathy; NARN- Naringenin; AG- Aminoguanidine
**Figure 6.1**: Effect of naringenin on physical parameters of control and experimental animals

The values that do not share a common superscript letter (a and b) between groups differ significantly at $p<0.05$ (Analysis of variance followed by DMRT; $n=10$).
6.1.2 Effects on kidney markers

Table 6.2, figure 6.2, 6.3, 6.4 and 6.5 shows the status of kidney markers in serum of the control and experimental groups. The concentration of serum creatinine, urea, uric acid and blood urea nitrogen (BUN) were increased significantly in group II animals as compared to control animals (group I). Oral administration of NARN were significantly decreased the levels of kidney markers. The serum analytes like potassium and sodium were increased in diabetic nephropathy induced group II animals compared with normal group. The altered analytes were normalized in NARN and AG treated animals. NARN (groups V) alone treated animals showed no significant difference in kidney markers as compared to control animals.

In diabetic nephropathy induced group II rats, the levels of serum total protein were significantly increased whereas decreased in albumin as compared to control animals. Oral administrations of NARN and AG to STZ administered animals revert back the status of total protein and albumin to near normal concentration. Rats treated with NARN alone showed no significant differences in serum total protein and albumin levels as compared to control animals.
Table 6.2: Effect of NARN on kidney markers (serum) in STZ-induced diabetic nephropathy.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II DN</th>
<th>Group III DN + NARN</th>
<th>Group IV DN + AG (Positive control)</th>
<th>Group V NARN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.80±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>22.65±2.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.31±5.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.51±3.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.10±3.82&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.13±3.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>15.21±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.18±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.12±0.97&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>19.12±1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.6583±1.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>73.16±9.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194.5±13.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.87±11.72&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>77.92±9.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.86±13.12&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>53.29±3.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.81±5.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.10±3.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.14±4.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61.27±3.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.57±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.03±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.36±0.30&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.97±0.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.12±0.22&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>121.85±6.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.02±7.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>137.19±7.47&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>129.12±7.04&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>125.02±6.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.40±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.76±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.78±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.50±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). DN- Diabetic Nephropathy; NARN- Naringenin; AG- Aminoguanidine
Figure 6.2: Effect of NARN on creatinine and urea (serum) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b, c, d and e) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
Figure 6.3: Effect of NARN on BUN and uric acid (serum) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b, c and d) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
**Figure 6.4:** Effect of NARN on total protein and albumin (serum) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b, c and d) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
Figure 6.5: Effect of NARN on sodium and potassium (serum) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b and c) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
The levels of kidney function markers in control and experimental animals showed in table 6.3, figure 6.6, 6.7 and 6.8. The concentration of creatinine, urinary urea nitrogen (UUN), glucose and albumin were significantly increased in group II animals. The altered kidney function markers were normalized in NARN and AG treated group of animals. Whereas the renal function analytes like potassium and sodium levels were increased in diabetic nephropathy which is considered a major complication of diabetes (table 6.3 and figure 6.8). Urine analysis of the STZ induced diabetic nephropathy rats showed also glucosuria and increased potassium, sodium, BUN, creatinine, uric acid, and albumin levels, which is a major complication of diabetes. Administration of NARN and AG significantly decreased the levels of kidney markers. NARN alone treated animals showed no significant difference in kidney markers as compared to control animals.
Table 6.3: Effect of NARN on kidney markers (urine) in STZ-induced diabetic nephropathy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II DN</th>
<th>Group III DN + NARN</th>
<th>Group IV DN + AG (Positive control)</th>
<th>Group V NARN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>27.02±1.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.05±4.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.13±2.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.43±2.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33.15±1.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary Urea Nitrogen(mg/dL)</td>
<td>124.02±6.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>630.43±34.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>422.49±23.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>325.06±17.71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>158.35±8.63&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>42.08±2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.27±10.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.03±5.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>99.03±5.93&lt;sup&gt;d&lt;/sup&gt;</td>
<td>52.02±3.12&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose(mg/dl)</td>
<td>22.12±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>198.38±11.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.45±5.42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.03±4.49&lt;sup&gt;e&lt;/sup&gt;</td>
<td>30.01±1.79&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium(mEq/L/day)</td>
<td>35.88±2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.26±3.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.02±2.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.01±2.39&lt;sup&gt;e&lt;/sup&gt;</td>
<td>37.74±2.26&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium (mEq/L/day)</td>
<td>70.08±4.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.15±5.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.63±4.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.55±4.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.14±4.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
DN- Diabetic Nephropathy; NARN- Naringenin; AG- Aminoguanidine
Figure 6.6: Effect of NARN on creatinine and urea nitrogen (urine) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b, c, d and e) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
Figure 6.7: Effect of NARN on albumin and glucose (urine) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b, c, d and e) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
Figure 6.8: Effect of NARN on renal function analytes (potassium and sodium) in STZ-induced diabetic nephropathy.

The values that do not share a common superscript letter (a, b and c) between groups differ significantly at p<0.05 (Analysis of variance followed by DMRT; n=10).
6.1.3 Renal histopathological studies

In histological examination, control (group I) samples of kidneys showed normal kidney morphology. STZ administered animals caused significant changes in tubular epithelium like vacuolization; desquamation, atrophy and necrosis; interstitial edema and inflammation in general architecture. But, administration of NARN and AG provided a well improvement in the renal morphology, it’s indicated that NARN has nephroprotective effects against DN. Tubular and glomerular structures were seen close to their normal structures in the NARN alone treated group.
Table 6.4: Histopathological changes in kidney tissues of normal and experimental animals

<table>
<thead>
<tr>
<th>Histopathological changes</th>
<th>Group I (Control)</th>
<th>Group II DN</th>
<th>Group III DN + NARN</th>
<th>Group IV DN + AG (Positive control)</th>
<th>Group V NARN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tubular epithelial desquamation</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tubular casts</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group.
DN- Diabetic Nephropathy; NARN- Naringenin; AG- Aminoguanidine
Quantification scores (-): no meaningful histopathologic change (+): mild degree; (++): moderate degree; (+++): severe degree
Figure 6.9: Photomicrographs of renal sections in group I animals.

Showing normal tubular architecture, tubules and glomerules that appear normal. [A&B-Haemotoxylin and Eosinstain X 100; C&D-Haemotoxylin and Eosinstain X 40].
Figure 6.10: Photomicrographs of renal sections in group II animals.

The epithelial cell vacuolization, desquamation, interstitial inflammation, luminal casts and dilatation of renal tubules are clearly observed. [A&B-Haemotoxylin and Eosinstain X 100; C&D-Haemotoxylin and Eosinstain X 40].
**Figure 6.11:** Photomicrographs of renal sections in group III animals.

Showing tubular epithelial desquamation, tubular atrophy and interstitial edema in some animals. [A&B - Haemotoxylin and Eosinstain X 100; C&D - Haemotoxylin and Eosinstain X 40].
Figure 6.12: Photomicrographs of renal sections in group IV animals.

Showing tubular necrosis, tubular epithelial desquamation, tubular atrophy and interstitial edema in some animals. [A&B - Haemotoxylin and Eosin stain X 100; C&D - Haemotoxylin and Eosinstain X 40].
Figure 6.13: Photomicrographs of renal sections in group V animals.

Showing normal tubular architecture, tubules, and glomerules that appear normal. [A&B - Haemotoxylin and Eosinstain X 100; C&D - Haemotoxylin and Eosinstain X 40].
6.1.4 Scanning Electron Microscopic (SEM) studies

Figure 6.14 demonstrate the photographs of scanning electron microscopic observation of control and experimental animals. The main observation is focused on the renal structure of glomerulus. Group I and NARN alone treated animals (Group V) showed normal structure of glomerulus, whereas STZ-induced animals (group II) exhibited shrinkage of glomerulus. Damaged renal tissues and parallel treatment with NARN (group III) and AG (group IV) animals elucidate better improvement of normal appearance and reduced in glomerulus shrinkage.
Figure 6.14: Scanning electron microscopic observation of kidney of control and experimental animals

A-Normal group I animals shows the normal architecture of glomerulus. B-STZ induced group II diabetic nephropathy animal shows loss of architecture and shrinkage of glomerulus. C – NARN treated animals shows reduced abnormal cells and maintaining near normal architecture of glomerulus (group III). D-NARN alone treated animals shows normal [Magnification 400X].
6.1.5 Transmission Electron Microscopic (TEM) studies

Figure 6.15 shows the transmission electron microscopic ultrastructural analysis of the kidney proximal tubule of control and experimental animals. The proximal tubule cells of control (group I) and drug alone treated rats (group V) contained a normal structure of brush border membrane, mitochondria, nucleus, basal infoldings and basal membrane. In the STZ-induced diabetic nephropathy (group II) animal’s proximal tubule epitomize loss of brush border membrane, loss of cristae in swollen or degenerative mitochondria, poorly developed basal infoldings and basal membrane were observed. The treatment with NARN and AG rats showed recovered proximal tubule cells of brush border membrane, mitochondria, nucleus, basal infoldings and basal membrane.
Figure 6.15: Transmission electron microscopic observation of kidney of control and experimental animals.

A – Group I control animals shows the proximal tubules with normal structure of brush border membrane, mitochondria, nucleus, basal infoldings and basal membrane [Magnification 10,000 X].

B - Group II STZ-induced DN animals shows a highly disturbed nucleus, disarrangement of cytoplasm loss of brush border membrane, loss of cristae in swollen, degenerative mitochondria, poorly developed basal infoldings and basal membrane [Magnification 15,000 X].

C - Group III NARN treated animals shows remarkable recovery in their tissue architecture, basal and brush border membrane and almost normal cytoplasm with protected number of a normal mitochondria [Magnification 15,000 X].

D - Group V NARN alone treated animals’ shows almost normal kidney tissue architecture with undisturbed nucleus, brush border membrane and cytoplasm [Magnification 15,000 X]. (BB-Brush Border Membrane, M-Mitochondria, N–Nucleus, BM-Basal Membrane).
6.2 DISCUSSION

Traditional herbal medicines have been widely used for the treatment of various diseases in the world. It has been reported that medicinal plants are used in folk medicine for the different diseases therapies and many of them are used for the therapy of DN [190-192]. Also there are several recent studies focusing positively on therapeutics of DN using traditional herbal medicines [193]. A series of bioactive compounds have been isolated from the medicinal herbs. Citrus fruits are considered to have renal protective activity. After thorough analysis, founds that naringenin was the major active ingredient in the citrus fruits.

This study adopted oral administration of NARN to determine the beneficial effects of it on improving hyperglycemia and DN. Hyperglycemia is a critical factor in the development of DN and is associated with an increase in mesangial cell proliferation and hypertrophy as well as increased matrix production and basement membrane thickening [194, 68]. Hence, glycemic control could slow the development of diabetic nephropathy [195]. In this investigation, NARN had a significant effect on limiting the increase in blood glucose levels which might be due to its hypoglycaemic effect [196].

DN is the common cause of leading to ESRD [197]. The symptoms of DN are characterized by kidney hypertrophy, increase in UAE, decreased kidney function, glomerulosclerosis and tubular interstitial fibrosis [198]. The accumulation of ECM is considered to be correlated closely with progression of DN [199] of group II animals. High glucose concentrations of STZ-induced DN rats stimulate the secretion of ECM leading to renal fibrosis and failure [200]. Therefore, searching for effective methods of inhibiting ECM accumulation may be of great importance for intervention in
progressive glomerulosclerosis in DN. NARN has been proposed as an effective agent in the therapy of DN.

The kidney weight index is an important indicator of the degree of kidney hypertrophy. Treatment with NARN (50 mg/kg b.wt/day) for 15 days attenuated kidney hypertrophy showed in group III animals. Body weight loss is commonly observed [201,202] in group II experimental DN animals. Body weight loss has been found to be related with muscle loss due to over catabolism of tissue proteins as a result of hyperglycemia [203]. In the present study, naringenin administered maintain the body weight by controlling blood glucose level thereby preventing protein catabolism. In the experimental diabetes studies, results on kidney weights are contradictory. Our studies show the kidney weight have increased like Garman et al [204] reports but Coldiron et al[201] reported decreases in kidney weights.

Albuminuria is a symbol of DN [205] and urinary albumin excretion is also certificated to be the best clinical predictor of renal lesions in DN [206] of STZ-induced animals. Creatinine is a breakdown product of creatinine phosphate in muscle and its clearance measured as an indicator of GFR. In this study, increase urinary albumin corresponding to the hyperglycemia after diabetes induction. Furthermore, levels of urine creatinine, kidney weight to body weight ratio, serum creatinine as well as BUN, urea, uric acid and albumin which are considered as markers of renal function, were higher in DN rats than those of normal rats. Continuous treatment with NARN (50 mg/kg b.wt/day) for 15 days obviously ameliorated all of those renal dysfunctions of DN progression in diabetic rats.

In DN rats, hyperglycemia induces the elevation of the serum uric acid, BUN and serum creatinine levels, which are deemed as the most sensitive markers of renal
dysfunction \cite{207,208}. Hyperuricemia exerts glomerular injury and mild tubulointerstitial kidney disease via uric acid-mediated endothelial dysfunction, activation of renin angio gensin system and induction of intrarenal inflammation \cite{209}. On the other hand, impaired balance of nitrogen coupled with lowered protein synthesis results in raised concentration of urea in blood \cite{210}. Increased serum uric acid, BUN and serum creatinine levels are denotations of the development of DN in rats \cite{192}. In addition, higher levels of serum uric acid and creatinine observed in the STZ-induced group II animals indicate the presence of DN with renal hyperfiltration \cite{211}. The increased BUN and serum creatinine in diabetic rats indicates progressive renal damage, which is taken as an index of altered GFR in DN \cite{212}.

Creatinine, the major waste product of creatine metabolism, is filtered by the glomerulus in the kidney and actively excreted by the tubules. Moreover, free creatinine appears in the blood serum \cite{213}, with high levels being indicative of muscle wastage \cite{214}. In the present investigation, the reversal of these elevated biochemical variables closer to near normal values by NARN supplementation in DN rats suggests its direct or indirect role, in offering protection against DN or delaying its development. This could be explained by a possible increased clearance of uric acid, blood urea and creatinine by the kidneys or reduced protein degradation.

The hyperglycemic effects of STZ-induced diabetes result in a model of DN that allows study using histopathology of the kidneys and serum BUN and creatinine levels \cite{215}. During the progression of diabetes, the elevation of the serum BUN and creatinine levels are due to the accumulation of ECM in the glomeruli and interstitium, which also leads to renal fibrosis known as DN \cite{216}. The accumulation of ECM and changes of renal structure after STZ-induced DN were observed.
However, treatment with NARN in DN rats was associated with improvements in glomerular fibrosis and renal structure.

The clinical hallmarks of diabetic nephropathy include glomerular basement thickening and mesangial expansion have been identified as pathological precursor of these clinical changes [217]. The loss of glomerular podocytes precedes and predicts the onset of clinical nephropathy and may be an early pathological manifestation of DN [218, 219] of group II rats. Podocytes are one of the important ingredients of filtration barrier which has special cytobiological characteristic and physiological function. The injury of podocytes can unavoidably lead to the occurrence of proteinuria [220].

Proteinuria led to a very early renal endothelial dysfunction in diabetes and accelerated the occurrence of tubular cell damage [221]. Therefore, reduction of proteinuria may be beneficial for kidney function and is able to prevent progression of DN towards the end-stage, namely, renal failure [222]. DN is characterized by a series of renal structure abnormality including basement membrane thickening, mesangial expansion, glomerulosclerosis and tubulointerstitial fibrosis [14]. Glomerular hyperfiltration, enlargement, mesangial expansion, and intertubular fibrosis can be found in diabetic kidney in association with an increase in the ECM [223] in group II STZ-induced DN animals.

The metabolic derangements in DN are often accompanied by sodium and potassium electrolyte imbalances [224]. This might be attributed to the hyperglycemic condition producing osmotic diuresis leading to marked urinary loses of water and electrolytes that might be exacerbated by urinary excretion of ketones compelling additional electrolyte loss [225]. With regard to sodium, there is extrusion through the
basolateral Na\(^+\)K\(^+\)-ATPase pump to the cytosol leading to decreased sodium pumping from the renal proximal tubules to the blood [226]. Also, many investigators have described the altered expression of renal sodium transporters in the collecting ducts and distal convoluted tubules in animal models of STZ-induced DN, leading to increased fractional excretion of sodium in urine [225].

Finally, renal morphology showed renal tubular lesions in DN (Group II) compared with normal rats (group I), although there was evidence of glomerulosclerosis. The renal tubule pathological changes in DN rats mainly comprised fatty or vacuolar degeneration (figure 6.10). Some studies have reported that tubulointerstitial injury is an important feature of DN, which can predict the renal dysfunction of early DN[227] and therapeutic interventions on renal tubule damage have been discussed in both the experimental and human settings[228].

The most common histopathological alterations including changes in tubular epithelium like vacuolization; desquamation, atrophy and necrosis; interstitial edema and inflammation in general architecture, capillary basal membrane thickening and diffused or nodular glomerulosclerosis on DN rats (group II) occur in glomerulus [229]. Diffused glomerulosclerosis is characterized by mesangial cell proliferation and expansion of mesangial matrix [230,231]. The administration of NARN and AG provided a well improvement in the renal morphology, it’s indicated that NARN has nephroprotective effects against DN. Tubular and glomerular structures were seen close to their normal structures in the NARN alone treated group.

Scanning Electron Microscopic (SEM) examination of kidney shows loss of architecture and shrinkage of glomerulus (figure 6.14B) as well as Transmission Electron Microscopic (TEM) examination showed highly disturbed nucleus,
disarrangement of cytoplasm loss of brush border membrane, loss of cristae in swollen, degenerative mitochondria, poorly developed basal infoldings and basal membrane in DN animals (figure 6.15B). Further, some diabetes-related alterations including loss of brush border, epithelial swelling or desquamation, accumulation of glycogen granules, and peritubular inflammation were detected. Some previous studies reported similar alterations on rats [232-235]. Glomerular and tubular changes were reduced in NARN and AG administered groups. Both SEM and TEM examination showed NARN evidently alleviated the progression of DN.

From this study, concurrent administration of NARN successfully prevented renal damage associated with DN, explored by various biochemicals, histological and ultra structural examinations. Alteration in mean body weight, BUN, creatinine and uric acid associated with DN were reduced by treating animals with NARN. The results of the present study indicate that NARN may emerge as a nephroprotective agent against DN.