Diabetic nephropathy is a clinical syndrome found in both type 1 diabetes and type 2 diabetes that is characterized by heavy proteinuria, renal failure, and arterial hypertension, with its hallmark being persistent albuminuria (>300 mg/24 hours) [278]. Its associated risk factors are high blood glucose, blood pressure, and cholesterol levels. Therefore, metabolic and hemodynamic factors should be controlled in order to prevent the occurrence of DN [364]. Hyperglycemia and hyperlipidemia are common in DN. There are reports that good blood glucose and serum lipid control can prevent DN [365,366].

Hyperlipidemia is a feature of STZ-induced diabetes in rats [367] as well as poorly controlled diabetes in humans [16]. The potential role of hyperlipidemia in progressive renal injury was proposed over a century ago [368]. More recently, experimental evidence suggests that lipids may be important modulators in the evolution of glomerular injury [369]. Increased dietary cholesterol promotes glomerulosclerosis in laboratory animals and a relationship exists in DN between increasing cholesterol levels and declining GFR [370]. However, in apparent conflict with these results, renal disease is infrequent in patients with primary hypercholesterolemia [371]. The explanation for this discrepancy is the additive effect of diseases in promoting renal injury. For example, when combined with other factors, such as hypertension, diabetes, or renal injury, the detrimental effects of hyperlipidemia on renal structure and function become consequential [372, 373]. In addition to being implicated in vascular injury and premature cardiovascular death, hypercholesterolemia and microvascular disease are cofactors with renal hypertension.
in promoting DN[374]. This interrelationship among several diabetic defects and sequelae may underlie the role of lipids in furthering renal damage [375].

Diabetic glomerulosclerosis entails an increase in mesangial matrix with much similarity to the pathophysiology seen in atherosclerosis. Thus, potentially glomerulosclerosis may be the renal manifestation of atherosclerosis and thus may contribute to the pathogenesis of DN. Dyslipidemia may also exacerbate DN by producing alterations in the coagulation-fibrinolytic system, changes in membrane permeability, and increasing atherosclerosis [376, 377]. Many clinical and experimental studies suggest the involvement of high levels of serum cholesterol in the development and progression of DN [77, 80].

Irrespective of type of lipid dysfunction leading to renal lipid accumulation, recent evidence suggests that altered lipoprotein metabolism and intracellular accumulation of unsaturated free fatty acids, cholesteryl esters, and advanced lipoxidation/glyoxylat ion end products can accelerate the development and progression of glomerular tubule-interstitial injury in patients with diabetes mellitus. Advanced lipoxidation/glyoxylat ion end products have been shown to promote the migration of proinflammatory and profibrotic monocytes / macrophages into the kidney [378].

The ancient Indians have identified and utilized the medicinal properties of several plants for the treatment of several diseases including kidney failure. Medicinal plants used in the folk medicine to treat several disorders including DN. However the antihyperlipidemic effect of NARN was not scientifically evaluated and therefore the present study was designed to investigate the hypolipidemic efficacy of NARN in STZ-induced DN.
9.1 RESULTS

Table 9.1, figure 9.1 and 9.2 shows the lipid and lipoprotein levels in serum of control and experimental animals in each group. The levels of total cholesterol, phospholipids, triglycerides, LDL-cholesterol, VLDL-cholesterol and c/p ratio were increased whereas the HDL-cholesterol levels were decreased in serum of STZ-induced DN animals as compared to control animals. Oral administration of NARN and AG were normalizing the altered levels of lipids significantly prevented hyperlipidemia in DN animals. Animals were treated with NARN alone showed (groups V) no significant difference in the levels of lipid and lipoproteins as compared to control animals.
Table 9.1 Effects of NARN on serum lipid profile in STZ-induced diabetic nephropathy rats

<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>Cholesterol (mg/dL)</td>
<td>145.05±8.69 a</td>
<td>278.09±16.65 b</td>
<td>165.14±9.89 c</td>
<td>180.71±10.82 d</td>
<td>160.10±9.59 c</td>
</tr>
<tr>
<td>Phospholipid (mg/dL)</td>
<td>24.75±1.71 a</td>
<td>36.41±1.96 b</td>
<td>26.20±1.67 a</td>
<td>30.18±1.64 c</td>
<td>28.68±1.51 c</td>
</tr>
<tr>
<td>c/p ratio</td>
<td>5.87±0.21 a/d</td>
<td>7.64±0.34 b</td>
<td>6.31±0.28 c</td>
<td>5.99±0.04 d</td>
<td>5.58±0.04 c</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>159.09±9.53 a</td>
<td>300.20±17.98 b</td>
<td>169.84±10.17 ac</td>
<td>175.80±10.53 c</td>
<td>160.06±9.59 a</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>53.08±2.90 a</td>
<td>33.02±1.98 b</td>
<td>48.11±2.88 c</td>
<td>47.91±2.61 c</td>
<td>51.91±3.11 a</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>31.82±1.91 a</td>
<td>60.04±3.60 b</td>
<td>33.97±2.03 ac</td>
<td>35.16±2.11 c</td>
<td>32.01±1.92 a</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>60.15±3.89 a</td>
<td>185.03±11.08 b</td>
<td>83.06±4.97 c/e</td>
<td>97.64±6.11 d</td>
<td>76.18±4.56 c</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 9.1**: Effects of NARN on serum cholesterol, triglycerides, phospholipids, HDL-cholesterol and VLDL-cholesterol in STZ-induced diabetic nephropathy rats.

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 9.2: Effects of NARN on serum LDL-cholesterol and c/p ratio in STZ-induced diabetic nephropathy rats.

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).
Table 9.2 and figure 9.3 shows the lipid levels in erythrocyte membrane of control and experimental animals in each group. The total cholesterol was significantly increased whereas phospholipids were slightly decreased in RBC membrane of STZ-induced animals as compared to control animals. Increased in c/p ratio was also observed in RBC membrane of group II animals compared with group I animals. Oral administration of NARN (50 mg/kg bwt for 15 days) and AG (100 mg/kg body weight; i.p) were normalized the altered levels of lipids significantly prevented hyperlipidemia of DN animals. Group V animals were treated with NARN alone showed no significant difference in the levels of lipids as compared to control animals.
Table 9.2 Effects of NARN on RBC membrane lipid profile in STZ-induced diabetic nephropathy rats.

<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>Total Cholesterol (mg/10^8 cells)</td>
<td>6.88±0.41^a</td>
<td>18.61±1.11^b</td>
<td>9.30±0.56^c</td>
<td>10.11±0.61^d</td>
<td>7.89±0.47^e</td>
</tr>
<tr>
<td>Phospholipid (mg/10^8 cells)</td>
<td>1.46±0.08^ad</td>
<td>1.24±0.07^b</td>
<td>1.33±0.07^bc</td>
<td>1.41±0.08^acd</td>
<td>1.50±0.08^a</td>
</tr>
<tr>
<td>c/p ratio</td>
<td>4.71±0.02^a</td>
<td>15.00±0.09^b</td>
<td>7.01±0.05^c</td>
<td>7.17±0.06^d</td>
<td>5.26±0.03^e</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 9.3: Effects of NARN on RBC membrane lipid profile in STZ-induced diabetic nephropathy rats.

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); * mg/10⁸ cells
The lipid levels in kidney tissues of control and experimental animals showed in table 9.3 and figure 9.4. The total cholesterol was significantly increased whereas phospholipids were slightly decreased in kidney tissues of STZ-induced animals as compared to control animals. Increased in c/p ratio was also observed in kidney tissues of group II animals compared with group I animals. Oral administration of NARN at the dose of 50 mg/kg bwt for 15 days and AG (100 mg/kg body weight; i.p) were normalized the altered levels of lipids significantly prevented hyperlipidemia of DN animals in group III and group IV respectively. Group V animals were treated with NARN alone showed no significant difference in the levels of lipids as compared to control animals.
Table 9.3 Effects of NARN on kidney tissues lipid profile in STZ-induced diabetic nephropathy rats

<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>Total Cholesterol (mg / g tissue)</td>
<td>3.93±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.62±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.22±0.27&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4.42±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.10±0.22&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phospholipid (mg / g tissue)</td>
<td>12.74±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.94±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.09±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.21±0.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.01±0.58&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>c/p ratio</td>
<td>0.31±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.086±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38±0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43±0.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.37±0.001&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 9.4**: Effects of NARN on kidney tissues lipid profile in STZ-induced diabetic nephropathy rats.

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)
9.2 DISCUSSION

Lipids are major cell membrane components, essential for various biological functions including cell growth and division of normal tissues. The abnormalities in lipid and lipoprotein pattern produce number of pathological diseases including DN. Kidneys are highly vulnerable to damage caused by ROS likely due to oxidative stress by polyunsaturated fatty acids in the composition of renal lipids [379]. Lipoproteins are responsible for the transport of lipids through the vascular and extra cellular tissue from their site of synthesis or absorption to peripheral tissues. An altered level of HDL-cholesterol, VLDL-cholesterol and LDL-cholesterol has been implicated in the pathogenesis of several diseases [380].

There is increasing evidence linking dyslipidemia as an independent contributing factor in the development and progression of glomerular injury, although the underlying mechanisms are currently debated. DN was known by not only dysfunctional glucose metabolism but also dyslipidemia, especially in type 2 diabetes. Hyperlipidemia promoted the development of DN [381] and moreover diabetes aggravated dyslipidemia [382], which create a vicious cycle ultimately. Hyperglycemia could enhance glomerulosclerosis and accelerate the progress of DN. Dysregulation of serum lipid parameters including total cholesterol, triglycerides, phospholipids, HDL-cholesterol as well as LDL-cholesterol, occurred in blood lipid metabolism disorder was commonly found in DN [383] animals (group II). *In vitro* or *in vivo* studies have strongly suggested that many biochemical and histological abnormalities seen in glomerulosclerosis are analogous to those observed in atherosclerosis [384] (chapter VI).
In the current investigation, DN animals have lipid profile abnormalities manifested by an increased level of total cholesterol, triglycerides and LDL-cholesterol and a decrease in serum HDL-cholesterol level. There is growing recognition of the importance of hyperlipidaemia and dyslipidaemia in the progression of microvascular disease in diabetes and the development of chronic renal disease [385]. Studies in humans have supported the relationship between dyslipidaemia and progression of chronic kidney disease [386,387]. Recently, Seliger [388] reported the association of dyslipidemia and inflammatory markers with decreased renal function among middle-aged and older type 2 diabetes,a population at high risk for chronic kidney disease. Treating dyslipidaemia can slow the progression of chronic renal disease [389].

Over two decades ago, Moorhead et al [390] noted an association between hyperlipidemia and glomerular capillary injury. The authors suspected that the persistent filtration of lipids and lipoproteins promote progression of chronic renal injury [390] in DN animals. Many subsequent observational studies supported the role of elevated levels of serum lipids in the development of albuminuria and in the progression of glomerulosclerosis [72, 371] Gall et al [72] reported a significant association between total serum cholesterol and increased urinary albumin excretion in DN animals. Animals with DN with higher serum and RBC total cholesterol and lower HDL-cholesterol developed a higher incidence of renal insufficiency [391]. The lipid profiles of group II animals with DN have been characterized to have higher serum concentrations of VLDL-cholesterol,LDL-cholesterol and triglycerides but lower levels of HDL-cholesterol. The aforementioned lipid profile has been termed “diabetic dyslipidemia”; it is mostly seen in individuals with type 2 diabetes.
Moreover, this is further characterized by a preponderance of dense, small-diameter LDL-cholesterol and HDL-cholesterol particles along with excessive postprandial lipemia, which results from increased concentrations of VLDL-cholesterol and chylomicron remnants [376, 392]. An increase in hepatic lipase activity and a reduced postheparin plasma lipoprotein lipase (LPL) ratio have also been documented [393].

Interestingly, the aforementioned lipid abnormalities in DN become more accentuated with worsening renal function and urinary albumin excretion (chapter VI) [373]. The lipid alterations stem from insulin resistance and a defective insulin action in the metabolism of lipoproteins. The net result consists of enhanced lipolysis with a subsequent increase in free fatty acids and VLDL-cholesterol synthesis, a defect in LPL activity leading to the increased life span of chylomicrons and VLDL-cholesterol in circulation, an increased transfer of cholesterol esters resulting in triglyceride-rich LDL-cholesterol, and finally the elevation of serum triglycerides and the reduced ratio of LPL to hepatic lipase causing the accelerated breakdown of HDL-cholesterol (Table 9.1 and figure 9.1 and 9.2) [392].

Nishida et al [394] found that LDL-cholesterol and TG rich lipoproteins (very low-density lipoprotein and intermediate density lipoprotein) caused proliferation of mesangial cells, whereas oxidized LDL-cholesterol had a cytotoxic effect on mesangial cells. It is possible that these actions are mediated via cytokines such as IL-6, PDGF, TGF-β, and tumor necrosis factor-alpha (TNF-α) of STZ-induced DN animals (chapter VII). Several underlying mechanisms by which dyslipidaemia may cause kidney damage and glomerular injury have been suggested. These mechanisms include, among others, endothelial dysfunction, macrophage activation and mesangial cell damage [395, 396].
In the tubulointerstitium, the renal injury due to hyperlipidemia has been suggested to be a prognostic indicator because tubulointerstitial lesions may precede glomerular changes and correlate better with renal disease progression [397] of group II animals were discussed in chapter VI. The tubular injury was ascribed to interstitial macrophage infiltration and an increase in TGF-β1 gene expression. It is believed that this is mediated via cytokine reactions and ROS (chapter VIII) [398, 399]. These phenomena are similar to those in vivo studies where the tubular uptake and metabolism of filtered lipoproteins resulted in the expression of cytokines and subsequent local inflammation [397, 400, 401].

In addition, hyperlipidemia participates in the progression of glomerular injury and a more rapid decline of renal function was observed in animals with DN in group II animals. Therefore, lipid-lowering therapy may have a beneficial effect of retarding the progression of DN. NARN has an impact on lipid metabolism in experimental animals (DN) inhibit the elevations in serum total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol levels, but it also reduced the increase in the blood glucose level [402] of group III animals, suggesting that NARN improves both carbohydrate and lipid metabolism. From the above results, we conclude that NARN improve lipid metabolism as well as glucose-dependent metabolism and thus we would expect it to protective activity against tissue damage induced by the metabolic disorders associated with DN.

Furthermore, DN the heavy renal deposition of lipids has been related to abnormal lipid metabolism, including down regulation of metabolic pathways for the removal of fatty acids, down regulation of renal lipoprotein lipase (LPL) for triglycerides hydrolysis, increased uptake of cholesterol and down regulation of genes
effecting cholesterol efflux. As a result, decreased glomerular filtration rate and inflammation correlate closely with dysregulation of lipid metabolism genes [403]. Another possible mechanisms accounting for renal lipid accumulations in DN animals were an increased activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), the key cholesterol biosynthesizing enzyme [404].

While the pharmacological stimulation of NARN resulted in upregulation of genes involved in cholesterol efflux, in down-regulation of proinflammatory cytokines, in inhibition of the pathomorphology of DN, in reduced formation of cytokines (chapter VII) and ROS (chapter VIII) and accumulation of renal lipids, and in improvement of renal function [378]. The results obtained in the current study indicate that NARN ameliorates hyperlipidemia caused by STZ-diabetic rats. Significant reductions were observed with NARN as regard lipid profile; serum total cholesterol (TC), TG and LDL-cholesteroland significant increase in HDL-cholesterol compared to STZ-induced DN rats. The rise in plasma TG levels in diabetic rats could result from decreased removal of circulating lipoproteins or from increased lipoprotein production and both mechanisms seem to be implicated in the genesis of the hypertriglyceridemia that occurs after STZ administration.

In DN, lipid deposits are evident in the glomerulus, renal tubule and renal vasculature [77]. Furthermore, mesangial expansion, characteristic of DN, may result from hyperlipidemia and elevated glomerular pressure [405] of group II animals. Proteinuria resulting from DN may promote dyslipidemia [406]. At the same time, reduction of serum lipid concentrations lowers proteinuria [371]. With respect to the linkage between proteinuria and dyslipidemia, increased urinary excretion of apolipoproteins and small lipoproteins may alter the synthesis of certain
apolipoproteins, thereby, changing the ratio of high to low density lipoproteins [407,408]. An alteration in the relative ratio of serum lipid components has far-reaching physiological effects. For example, relative increases in concentrations of free, circulating LDL-cholesterol can bind polyanionic glycoproteins in the GBM [390]. Such binding decreases membrane selectivity, resulting in proteinuria and deposition of lipoprotein, lipid and other molecules in the mesangium [390]. The presence of these molecules in the mesangium could stimulate mesangial proliferation [409]. Thus, dyslipidemia produces renal changes characteristic of DN. There are similarities between mechanisms of lipid injury to the glomerulus and atherosclerosis [370]. While atherosclerosis plays a role in proteinuria and DN, the converse is also true [370, 410]. Alterations in relative concentrations of serum lipoproteins and apolipoproteins caused by increased protein excretion play a role in the genesis of atherosclerosis [370]. Even with modest proteinuria in the microalbuminuria range, an atherogenic lipid profile is present [370]. Animals with DN (group II) exhibit higher serum TG concentrations than animals (group I) without DN [411].

The study demonstrating that hyperlipidemia and hyperglycemia act synergistically to induce renal injury in group II animals has further indicated that lipid can exacerbate DN. Oral administration of NARN brought back the values to near normal range in STZ-administered rats. The results of the present study indicate that NARN may emerge as a putative antihyperlipidemic agent against DN.