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Review Article

# An Overview of Diabetic Nephropathy

**Rajavel Varatharajan[1,2*], Jaganathan Saravanan[3], Ivy Chung[2] and Nor Azizan Abdullah[2]**

[1]Unit of Pharmacology, Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia.
[2]Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3]Department of Pharmaceutical Chemistry, School of Pharmacy, Kuala Lumpur Metropolitan University College, Jalan Raja Laut 50350, Kuala Lumpur, Malaysia.

## ABSTRACT

Diabetic nephropathy is one of the common complications of diabetes mellitus that has become the leading cause of end-stage renal failure in many developed countries. In general, 1 of 3 diabetic patients is developing diabetic nephropathy. High blood pressure, high cholesterol and smoking are increasing the risk of development of diabetic nephropathy. Many factors involving in the development of diabetic nephropathy, which includes oxidative stress and non-enzymatic sources of oxidative stress. In early stage, diabetic nephropathy has no symptoms and it make take 5-10 years to appear the kidney damage begins. Glomerular basement membrane thickening and mesangial expansion are the main morphological features of the diabetic nephropathy. This can be treated or modified with angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists and antioxidants. The main objective of this review is to discuss about the pathogenesis and pharmacotherapy of diabetic nephropathy.

**Key words:** Angiotensin-Converting Enzyme Inhibitors, Antioxidants, Diabetic nephropathy, NADPH oxidase, Oxidative stress.

## INTRODUCTION

Diabetic nephropathy is one of the common complications of diabetes mellitus that has become the leading cause of end-stage renal failure in many countries.[1] Ingeneral, about 1 out of 3 patients with Type 1 Diabetes Mellitus (T1DM)or Type 2 Diabetes Mellitus (T2DM) proceed to developing significant diabetic nephropathy.[2] The pathophysiologic mechanisms of renal disorder might be similar in both types of diabetes mellitus.[3] The pathogenesis and clinical course of diabetic nephropathy can be monitored by renal structuraland hemodynamic changes. The earliest change is an increase in glomerular filtration rate(GFR), followed by detectable glomerular lesions. This could follow with the development of microalbuminuria. Once microalbuminuria persists, both changes in glomerular structure, such as mesangial expansion and basement membrane thickening, and permeability could occur. At this stage, there are progression prominent proteinuria, hypertension, and renal insufficiency. The pathological alterations in this stage are glomerular basement membrane thickening, and mesangial expansion, resulting indiffuse and/or nodular glomerulosclerosis and tubulointerstitial fibrosis.[4] After long time of persistentproteinuria, progression to end-stage renal disease could occur.[5] In addition, the advanced diabetic glomerulopathy is commonly characterized by diffuse glomerulosclerosis and may sometimes exhibit a distinctive morphological appearance, namely, the nodularform of glomerulosclerosis, as first described by Kimmelstiel and Wilson in the year 1936.[3,6] Table 1 shows the stages of diabetic nephropathy.[7] The current strategies to treat diabetic nephropathy include intensive glycaemic control, antihypertensive treatment with a particular focus on renin-angiotensin-aldosterone-system (RAAS) interruption, dietary protein restriction, and hyperlipidemia treatment. There are several hypothetical approaches to the treatment of diabetic nephropathy based on an evergrowing mechanistic understanding of the causes of diabetic nephropathy. These approaches include pharmacologic inhibitions of Advanced glycation endproducts (AGEs) formation, oxidative stress, protein kinase C (PKC), and transforminggrowth factor β (TGF-β) in the kidney.[8] Some medicinal herbs have been used widely for the treatment of diabetes and diabetic complications for hundreds of years.[9,10]

**Corresponding Author :**
**Mr. Rajavel Varatharajan**
Unit of Pharmacology, Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia.
Ph no: 0060-173166982, Fax no: 006 044268132
E-mail: varadharajeen@gmail.com

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## Features of Diabetic Nephropathy

**Glomerular Basement Membrane Thickening:** Glomerular basement membrane (GBM) thickening is a characteristic early change in T1DM[11-13] and T2DM[14] and occurs with duration of disease.[15] Glomerular basement membrane thickening is a consequence of Extracellular matrix (ECM) accumulation, with increased deposition of normal ECM components such as collagen, laminin, and fibronec-

**Table 1: Stages of diabetic nephropathy in T1DM**

| Stage | Renal Manifestation |
|---|---|
| 1 | Renal hyperfiltration (GFR↑)<br>Renal hypertrophy |
| 2 | Silent stage Renal hyperfiltration; normal urine albumin excretion rate<br>Early histologic changes; non-specific increase in basement membrane thickness and increase mesangial matrix |
| 3 | Microalbuminuria (UAER 30-300mg/24h) or incipient nephropathy<br>GFR may elevate or reduced into normal range<br>Histology: mesangial expansion, glomerular basement membrane thickening and arteriolar hyalinosis |
| 4 | Established or overt nephropathy (proteinuria, nephrotic syndrome)<br>GFR decline and hypertension |
| 5 | End stage renal disease |

tin.[16,17] Such accumulations could result from either increased production of these proteins or their decreased degradation, or both. Glomerular basement membrane thickening might already be present in T1DM patients, who are normoalbuminuric.[13,15]

**Mesangial Expansion:** Mesangial cells are found in a part of the kidney called the glomerulus involved in filtration in the urine. Water, waste, and excess nutrients are filtered from the blood through the capillary walls into the surrounding Bowman's capsule. The mesangial cells are found between the capillaries and help regulate the filtration process while providing support for the glomerular structure and they are involved in the kidney's response to injury and disease. Intra-glomerular mesangial cells have an irregular shape and are related to smooth muscle cells. They do have similar proteins such as myosin and actin, and have the ability to contract.

## Pathogenesis of Diabetic Nephropathy

As described previously, diabetic nephropathy is a major cause of renal failure. Numerous factors have been implicated in the pathogenesis of diabetic nephropathy that include association of genetic factors, haemodynamic factors, vasocoactive factors, growth factors, renal structural factors, and hyperlipidemia. Among these, it has been confirmed that chronic and uncontrolled hyperglycemia plays a significant role (Figure 1), and it is the major culprit in the development of diabetic nephropathy.[18]

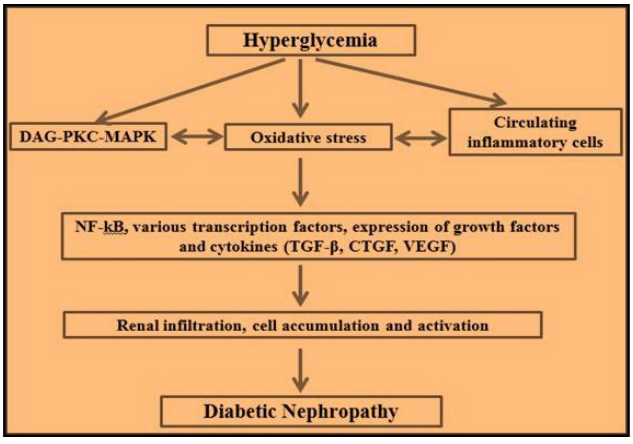


**Figure 1: The induction and progression of diabetic nephropathy[19]**

CTGF-Connective tissue growth factor; DAG-Diacylglycerol; MAPK-Mitogen-activated protein kinases; PKC-Protein kinase; TGF-β Transforming growth factor beta; VEGF-Vascular endothelial growth factor.

**Oxidative stress:** In 1956, Denham Harman, father of the free radical theory, suggested that free radicals produced during aerobic respiration cause cumulative oxidative damage.[20] Free radicals are generally considered harmful byproducts of oxidative metabolism,[21] causing organ damage in living systems. This concept has implications in numerous biological phenomena such as cellular aging, mutagenesis, inflammation, and other pathologies.

Oxidative stress is defined as the excess formationor insufficient removal by antioxidant defenses of highly reactive molecules including ROS and Reactive nitrogen species (RNS). Examplesof ROS include free radicals, superoxide($O_2^{-}$), hydroxyl (HO·), peroxyl ($O_2$) and hydroperoxyl($HO_2$). Examples of RNS include peroxynitrite($ONOO^{-}$) and alkyl peroxynitrates (RONOO). The major free radical implicatedin diabetic complications is superoxide, which could be formed by various sources, like the mitochondrial Electron transport chain (ETC) during oxidative phosphorylation, NADPH oxidase, cyclooxygenase, lipoxygenase, xanthine oxidase, cytochrome P-450, and nitricoxide synthase in certain contexts.[22] In the presence of transition metalssuch as iron and copper, $H_2O_2$ can be converted to the highly reactive HO· radical via the Fenton reaction. Excess $O_2^{\cdot-}$ also can reactwith NO· to form $ONOO^{-}$.[22]

In normal conditions, $O_2^{\cdot-}$ is eliminated rapidly by antioxidant defense mechanisms. Superoxide dismutase (SOD) can catalyze the dismutation of $O_2^{\cdot-}$ to $H_2O_2$. SOD has 3 major isoforms: cytosolic CuZn-SOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular SOD (SOD3). $H_2O_2$ is converted to $H_2O$ and $O_2$ via catalase in lysosomes or glutathione peroxidase (GPx) in the mitochondria and cytosol.

## Non-enzymatic Sources of Oxidative Stress

It is widely recognized that oxidative stress is a key component in the development of diabetic complications. Non-enzymatic sources of oxidativestress induced by diabetes mellitus include glucose auto-oxidation, advanced glycation, the polyol pathway, and the mitochondrial ETC[23] (Figure 2). It has been suggested that the primary initiating event in the development of diabetic complications is $O_2^{\cdot-}$ formation by mitochondria.[24] Hyperglycemia induces changes in the mitochondrial voltage gradient by increasing electron donors of the ETC (Nishikawa *et al*., 2000). Diabetic rats have mitochondrial enlargement in renal proximal tubules associated with disturbed ATP metabolism.[25] Furthermore, diabetic rats have altered renal mitochondrial permeability transition.[26]

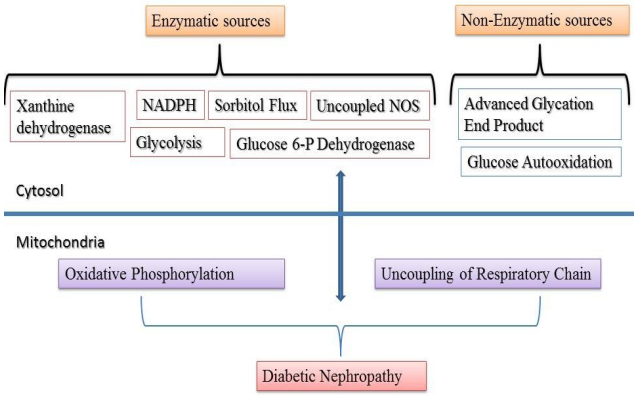


**Figure 2: Cytosolic and Mitochondrial sources of ROS implicated in the pathogenesis of DN[27]**

## Enzymatic sources of oxidative sources-NADPH Oxidase

Nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidases are known to generate Reactive oxygen species (ROS). NADPH oxidase (NOX)-1 and NOX-2 are major sources of ROS, and are shown to be involved in the pathogenesis of endothelial dysfunction in early stages of diabetic nephropathy.[28] NADPH oxidases

are composed of six subunits including two membrane-associated components (p22 phagocytic oxidase (phox) and gp91phox) and four cytosolic components ( and small GTPase Rac). NADPH oxidase is activated by membrane translocation of three cytosolic proteins (p47phox, p67phox, and small GTPase Rac). NADPH oxidases catalyse the transfer of electrons from the cytosol across biological membranes and into various intracellular and extracellular compartments.[29] At the biological membrane, these proteins assemble with gp91phox-p22phox heterodimer and induce a conformational change of gp91phox, resulting in superoxide production.[30] A large and rapidly expanding body of experimental evidence implicates NADPH oxidases in the constitutive cells of the artery wall, as underlying causes of oxidative stress in various micro-vascular diseases, including nephropathy.[31-33]

The NADPH oxidase activation is a key pathogenic mechanism involved in the induction and progression of diabetic nephropathy.[34] An increased understanding of the role of oxidative stress in the pathogenesis of diabetic nephropathy has lead to the exploration of a number of anti-oxidant strategies to treat diabetic nephropathy.

## Role of NADPH Oxidase in the Pathogenesis of Diabetic Nephropathy

It has been suggested that free radicals are implicated in the development of diabetic microangiopathy and macroangiopathy.[35] Excessive free radical production has been reported in diabetics with chronic renal failure treated by haemodialysis.[36] Consequently, free radical mechanisms have been implicated in the pathogenesis of tissue damage in diabetes.[21,37-39] Diabetic nephropathy is characterized by excessive deposition of ECM in the kidney, leading to glomerular mesangial expansion and tubulointerstitial fibrosis.[40] NADPH oxidase activation-induced oxidative stress is involved in the pathophysiology of various vascular diseases.[41]

Oxidative stress-mediated vascular endothelial dysfunction could play a central role in the pathogenesis of diabetic nephropathy.[42] Oxidative stress is apparent during the stage of glucose intolerance long before clinically apparent diabetes mellitus. The NADPH oxidase pathway constitutes the most important source of ROS in individuals with diabetes mellitus.[43] Likewise, hyperglycemia-induced oxidative stress has been shown in patients with clinically established diabetic mellitus.[44] Importantly, NADPH oxidase is a cytosolic enzyme complex located in the plasma membrane of various renal cell types, such as including mesangial and proximal tubular cells, endothelial cells, vascular smooth muscle cells, and fibroblasts.[45,46] This indicates the involvement of NADPH oxidase in the detrimental diabetic renal milieu. Hyperglycemia can trigger the activation of the RAAS in patients with early T1DM.[47] Angiotensin-II (Ang-II), an executive peptide of the RAAS, is one of most potent inducers of NADPH oxidase and markedly contributes to ROS generation in diabetic nephropathy.[28] Hence, it may be considered that angiotensin II can directly activate NADPH oxidase in diabetic nephropathy. In the kidney, the primary role of the NADPH complex is as a signalling molecule. The enhanced PKC-mediated NADPH oxidase activation by Ang-II or transforming growth factor β (TGF-β) leads to excessive generation of free radicals, inducing renal hypertrophy in rats and other detrimental effects renal effects.[31]

The aforementioned evidences provide a rationale for the use of pharmacological inhibitors of NADPH oxidase to reduce oxidative stress and its associated vascular pathologies in diabetic nephropathy. Among various available inhibitors, apocynin and diphenyleneiodonium (DPI) are the most widely studied inhibitors. Other than apocynin and DPI, taurine is also noted as important NADPH oxidases inhibitor. The frequent albuminuria and development of glomerulopathy in diabetic rabbits was significantly attenuated by the treatment with taurine. Thus, the nephroprotective effect of taurine could be attributed to its NADPH oxidase inhibitory property.[48] It is noteworthy that apocynin restored endothelial dysfunction in diabetic rats through regulation of nitric oxide synthase and NADPH oxidase expression.[49] Further, apocynin effectively blocked high glucose-induced ROS generation in mesangial cells.[8] Moreover, treatment with apocynin markedly attenuated the renal oxidative stress induced via NADPH oxidase and prevented the development of diabetic nephropathy by decreasing the renal expression of fibronectin and collagen-I in diabetic rats.[50] Furthermore, inhibition of NADPH oxidase using apocynin attenuated the progression of diabetic nephropathy in rats by reducing the occurrence of albuminuria and preventing the development of glomerulosclerosis.[51] The other injurious role of NADPH oxidase in diabetic nephropathy via occurrence of albuminuria by renal oxidative stress and glomerular expression of vascular endothelial growth factors in Otsuka Long Evans Tokushima Fatty (OLETF) rats was significantly attenuated with the use of apocynin.[52]

Under diabetic conditions NADPH oxidases played a key role in methylglyoxal-induced renal fibrosis via superoxide generation.[53] Herbal drugs have wide spread utility and they allege as antioxidant in various microvascular diseases. Continually, administration of green tea, an antioxidant, downregulated NADPH oxidase in diabetic spontaneously hypertensive rats and significantly attenuated the development of nephropathy.[54] Since diabetes mellitus-mediated activation of NADPH oxidase induces oxidative stress, which is involved in renal damage, targeting NADPH oxidase is considered as an important therapeutic option to prevent development of diabetic nephropathy.

## Molecular Mechanisms of Oxidative Stress in Diabetic Nephropathy

Four major biochemical pathways are considered to lead to the development of diabeticcomplications associated with hyperglycemia: (a) the polyol pathway: glucose is converted to sorbitol and subsequently metabolized to fructose (b) the hexosaminepathway, fructose-6-phosphate is converted to intermediates of glucosamine and the ROS productionis increased, (c) the protein kinase C (PKC) pathway, glucoseis converted to glyceraldehyde-3-phosphate, leading to the formation of diacylglycerol(DAG). The elevation of intracellular DAG levels activate PKC, and then activate NADPH oxidase to induce ROS formation, (d) theformation of advanced glycation end products (AGEs), interactionof AGEs with the receptors of advanced glycation end-products (RAGE) results inROS activation (Figure 3).[27,55-61]

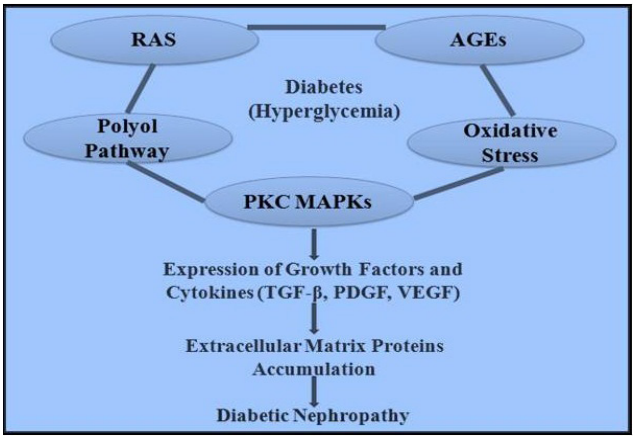


**Figure 3: Biochemical hypothesis for diabetic nephropathy[55]**

Increased oxidative stress has been widely involved in the development and progression of diabetes mellitus and its complications including diabetic nephropathy.[62] ROS are generated inglomerular mesangial and tubular epithelial cells by high glucose, AGE, and cytokines.[63] Excessive amount of free radicals induce damage to membrane lipids cellular proteins, nucleic acids, and then cause cell death.[62] In addition increased ROS can cause vascular endothelium abnormalities, reacting directly with Nitric oxide (NO) to produce cytotoxic peroxynitrite and increasing reactivity to vasoconstrictors and modification of ECM proteins.[64] The oxidative stress can also damage endothelial cells indirectly by stimulating expression of various genes involvedin inflammatory pathway.[65] Previous study finds that high glucose induces ROS and then up-regulates TGF-β1 and

ECM expression in the glomerular mesangial cell.[8] Ha[66] *et al.* (2002) reported that ROS mediates high glucose-induced activation of NF-κB and NF-κB dependent expression of monocyte chemoattractant protein (MCP)-1. A nuclear transcription factor NF-κB could initiate the gene transcription associated with inflammatory response, induced by many cell stress-associated stimuli like cytokines, vasoactive agents, growth factors and oxidative stress (Kuhad and Chopra, 2009). Increased steady-state mRNA levels of inflammatory genes have been shown to associate with interstitial fibrosis and progressive human diabetic nephropathy.[67]

Transforming growth factor-β, a fibrotic cytokine that is up-regulated by ROS, plays an important role in the development of renal hypertrophy and accumulation of ECM components in diabetes mellitus.[68] The TGF-β expression was found increased in diabetic nephropathy.[69-72] Anti-TGF-β antibody treatment has been documented to attenuate the effect of high glucose-induced cellular hypertrophy *in vitro* and in streptozotocin-induced diabetic mice.[73-75] TGF-β is also the key regulator of ECM remodelling in mesangium causing mesangial expansion and inducing the process of epithelial-mesenchymal transition (EMT) causing tubulointerstitial fibrosis.[76,77] There are also evidences that antioxidants can effectively inhibit high glucose-induced TGF-β1 and fibronectin up-regulation.[78]

Although strict glycemic control is very important in diabetic patients, many of the currenttherapeutic approaches might ameliorate oxidative stress as pleiotropic effects,[60] including angiotensin converting enzyme (ACE) inhibitors,[79] angiotensin II $AT_1$ receptor blockers (ARBs)[80] and aldosterone receptor antagonist (spironolactone).[81] They activate eNOS, increase NO bioavailability, inhibit the synthesis of Ang-II and TGF-β and to decelerate or prevent tubulointerstitialfibrosis in diabetic nephropathy, accompanied with control of systemic andintra-renal blood pressure.

AGE products contribute to the pathogenesis of diabetic nephropathy via receptor mediated mechanisms and indirectly via the generation of ROS. Circulating levels of AGE products in diabetic patients were elevated with decreased renal function.[82] In addition, AGE accumulation in tissues correlated with the severity of organ injury, particularly with glomerular lesions.[83,84] In the development of diabetic nephropathy, excesses of AGEs such as pentosidine and carboxymethyllysine have been identified in the expanded mesangial area and thickened glomerular capillary wall.[85,86] *In vitro*, AGE products have been shown to increase TGF-β1 and ECM expression in glomerular endothelial and mesangial cells thatwas enhanced in hyperglycemic conditions.[87-90]

Dietary AGE products are also thought to contribute to the development of diabetic nephropathy. Moreover, diets high in AGE content were known to impair insulin sensitivity.[91] Various agents, including LR-90,[92] aminoguanidine[93] and alagebrium chloride alagebrium chloride (ALT-711)[94] were potent in reducing AGE accumulation in renal tissues in experimental diabetic nephropathy, and subsequently improving renal function. Benefits were also seen in the clinical context with agents such as metformin, which decrease toxic dicarbonyls and AGE products in addition to its anti-hyperglycemic action.[95] Furthermore, benfotiamine (liposoluble vitamin B1 derivative), decreased AGE accumulation, inflammation and improved vascular function in T2DM patients consuming diets high in AGE content.[56]

Although numerous signaling mechanisms have been identified to be involved in the pathogenesis of diabetic nephropathy, a precise signaling pathway with complete picture is yet to be identified. Current studies are therefore focusing to identify major signaling culprits prominently involved in the pathogenesis of diabetic nephropathy. Studies till date support with strong evidences that renal oxidative stress due to activation of NADPH oxidase plays a pivotal role in the pathogenesis of diabetic nephropathy.

## Pharmacotherapy of DN

There is no satisfactory therapeutic option is currently available to treat patients with nephropathy except for littler agents like angiotensin converting enzyme inhibitors, angiotensin AT1 receptor blockers and few antioxidants, which have been shown to improve the function of diabetic kidney to some level. Thus, marvelous efforts are being made to explore promising therapeutic mediations to treat diabetic nephropathy. Telmisartan (80 mg/day) was not lesser to enalapril (20 mg/day) in preventing the progression of decline of GFR in type 2 diabetic patients with microalbuminuria, reinforcing the recommendation that ACE inhibitors and ARBs have a similar effect in protecting the kidney damage. The role of ACE inhibitors in the prevention of diabetic nephropathy in patients with type 1 diabetes has not been defined. The use of perindopril during 3 years in normotensive normoalbuminuric type 1 diabetic patients delayed the increase in albuminuria. In patients with type 2 diabetes, ACE inhibitors and ARBs both diminish the risk for diabetic nephropathy and reduce the occurrence of cardiovascular events. In the MICRO-HOPE (Heart Outcomes Prevention Evaluation) study ramipril (10 mg/day) decreased the risk of overt nephropathy by 24% and the risk of cardiovascular death in patients with type 2 diabetes who were >55 years of age with one additional cardiovascular risk factor by 37%. Moreover, ramipril reduced UAE at 1 year and at the end of the study. Therefore, ACE inhibitors have been shown to be beneficial for reno-and cardioprotection in patients with type 2 diabetes.[96,97]

## CONCLUSION

In the last few years, enormous progress in the understanding of the risk factors and mechanisms of diabetic nephropathy, the stages of renal involvement in diabetes, and the treatment strategies to prevent the progression of diabetic nephropathy. Early detection of diabetic nephropathy, adoption of multifactorial interventions targeting the main risk factors (hyperglycemia, hypertension, dyslipidemia, and smoking), and use of agents with a reno-protective effect (ACE inhibitors and/or ARBs) do indeed reduce the progression of renal disease. DN remains the leading cause of ESRD in developed countries, and its occurrence seems to be aggregate in the developing countries. The cost of treating this situation, mainly when patients require renal replacement therapy is huge in order to attain the healthy people 2020 goal. Multifactorial tactic directing strict control of blood sugar, lipids and blood pressure and use of ACEIs and ARBs in proteinuric patients will be preferable. Moreover in addition, low cost community-based program to increase physical activity and avoid unhealthy lifestyle. This will leads to decline in the incidence of diabetes in the population.

## CONFLICT OF INTEREST

No conflict of interest is declared.

## ABBREVIATION

| | |
|---|---|
| T1DM: | Type 1 Diabetes Mellitus |
| T2DM: | Type 2 Diabetes Mellitus |
| GFR: | Glomerular Filtration Rate |
| RAAS: | Renin-Angiotensin-Aldosterone System |
| AGEs: | Advanced Glycation End Products |
| PKC: | Protein Kinase C |
| TGF-β: | Transforming Growth Factor β |
| GBM: | Glomerular Basement Membrane |
| ECM: | Extracellular Matrix |
| RNS: | Reactive Nitrogen Species |
| O2.⁻: | Superoxide |
| HO.: | Hydroxyl |
| O2-: | Peroxyl |
| $HO_2$.: | Hydroperoxyl |
| $ONOO^-$: | Peroxynitrite |
| RONOO: | Alkyl Peroxynitrates |
| ETC: | Electron Transport Chain |
| SOD: | Superoxide Dismutase |
| GPx: | Glutathione Peroxidase |
| ROS: | Reactive Oxygen Species |
| Phox: | Phagocytic Oxidase |

| | |
|---|---|
| DPI: | Diphenyleneiodonium |
| OLETF: | Otsuka Long Evans Tokushima Fatty |
| DAG: | Diacylglycerol |
| NO: | Nitric Oxide |
| MCP: | Monocyte Chemoattractant Protein |
| EMTP: | Epithelial-Mesenchymal Transition |
| ACE: | Angiotensin Converting Enzyme |
| ARBs: | Angiotensin Receptor Blockers |
| ALT-711: | Alagebrium Chloride Alagebrium Chloride |

## Highlights of Paper

- Diabetic Nephropathy is one of the common complications of Diabetes mellitus.
- In general, 1 of 3 diabetic patients is developing diabetic nephropathy.
- Glomerular basement membrane thickening and mesangial expansion are the main morphological features of the diabetic nephropathy.
- Nicotinamide adenine dinucleotide phosphate reduced oxidases are known to generate reactive oxygen species.
- NADPH oxidase (NOX)-1 and NOX-2 are major sources of ROS, and are shown to be involved in the pathogenesis of endothelial dysfunction in early stages of diabetic nephropathy.
- This can be treated or modified with angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists and antioxidants.

## Author Profile


- **Mr. Rajavel Varatharajan:** Presently working as an Assoc. Prof. in the Pharmacology Unit, Faculty of Pharmacy, AIMST University, Kedah, Malaysia. He has more than 9 years of teaching experience. Published more than 25 articles and attend varies conference and workshop in international level.

- **Mr. Jaganathan Saravanan:** Presently working as lecturer in Department of Pharmaceutical Chemistry, School of Pharmacy, Kuala Lumpur Metropolitan University College, Kuala Lumpur, Malaysia. He has published more than 10 articles in varies journals and attend few workshop and conference in International levels.

- **Dr. Ivy Chung:** Presently working as Assoc. Prof. in Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. She has published more than 15 articles and attend varies conference and workshop in international level.

- **Dr. Nor Azizan Abdullah:** Presently working as Professor in Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. She has published more than 50 articles and attend varies conference and workshop in international level.




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