INTRODUCTION

Diabetes mellitus, a metabolic disorder associated with various macrovascular and microvascular complications including nephropathy. Nephropathy is one of major microvascular complication associated with the chronic diabetes mellitus. The uncontrolled glucose level results in structural as well as functional changes in the kidney. The common structural changes such as thickening of glomerular basement membrane, glomerulosclerosis, glomerular hypertrophy, podocyte loss, mesangial cell expansion, and tubulointerstitial fibrosis. After numerous afford it has been noted that the multiple signaling pathways are involved in the pathogenesis of diabetic nephropathy which include angiotensin-II (ANG-II), endothelin-1 (ET-1), advanced glycation end products (AGEs), endothelial nitric oxide synthase (eNOS), ROS, transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), nuclear factor kappa B (NF-κB), lipid peroxidation, and collagen-4 but it is worthwhile to note that ROS-RNS mediated oxidative stress through Nicotinamide adenine dinucleotide phosphate (NADPH) overproduction seems to be a centrally involved in the pathogenesis of diabetes induced nephropathy. Major ROS & RNS consist of paramagnetic free radicals (superoxide O$_2^-$, hydroxyl OH, peroxy radical ROO, nitric oxide free radical, diamagnetic molecules (H$_2$O$_2$ and peroxynitrite ONOO$^-$) which are products of the reactions of these free radicals. Thereby, the objective of this review is the suppression of damaging ROS-RNS signalling in biological processes whereby ROS-RNS induced by NADPH play a major role in pathogenesis and progression of diabetes induced nephropathy.

NADPH OXIDASE

NADPH oxidase is a critical component for ROS generation in macrophages and neutrophils and many other cell including mesangial cells. NADPH oxidase, a major source of superoxide generation, is composed of six subunits including two membrane associated components (p22 phagocytic oxidase (phox) and gp91phox) and four cytosolic components (P47phox, p40phox, p67phox, and small GTPase Rac). NADPH oxidase is activated by membrane translocation of three cytosolic proteins (p47phox, p67phox, and small GTPase Rac). At the membrane, these proteins assemble with gp91phox-p22phox heterodimer and induce a conformational change of gp91phox, which results in superoxide production. The activated NADPH oxidase generates superoxides by transferring an electron inside the cells across the membrane, and the electrons thus transferred are coupling with molecular oxygen to generate superoxides, known as reactive free radicals$^3$. NADPH is formed during glycolysis or oxidative phosphorylation and exerts antioxidant activity by regeneration of reduced glutathione. Glutathione act as important intracellular antioxidant by reacting with ROS and organic peroxides$^{2,3}$. Thus, antioxidant defense system will reduce the level of NADPH by conversion of NADPH to NADP$^+$. In renal vessels, macula densa, thick ascending limb of loop of henle, distal tubules, collecting ducts, interstitial fibroblasts, glomerular podocyte and messengial cells are documented to consist of enzyme NADPH oxidase which is responsible for significant production of superoxide radical$^4$. Activation of NADPH oxidase may be due to assembly of the subunits and translocation of p47phox to the membrane is necessary.

NADPH OXIDASE INDUCE NOS

Nitric oxide (NO) production plays a central role in modulating endothelial function$^5$. NO is generated from the metabolism of L-arginine by the enzyme NOS, of which there are three isoforms: the constitutive types, brain (bNOS) and endothelial (eNOS), and the inducible type (iNOS)$^6$. iNOS is induced de novo by various stimuli, including hyperglycemia$^7$, while the mitochondrial-generated superoxide can inhibit eNOS, although enough NO is still produced$^8$. NADPH oxidase generated superoxide radicals can react with NO forming peroxynitrite, which is a potent oxidant and nitrosylating agent. Furthermore, this reaction can cause NO deficiency, NO normally regulates tubuloglomerular feed back and renal blood flow, and is involved in regulation of natriuresis. The NO deficiency can be worsened by the fact that oxidative stress promotes activation of vasoconstrictors$^9$. Moreover, NO deficient animal models have shown to develop glomerulosclerosis and proteinuria, as well as hypertension and renal failure$^9$. Expression of p47phox is increased in podocytes, glomeruli, loop of henle, cortical distal tubules, and medullary collecting ducts in diabetic rats$^{10,11}$. Further NADPH oxidase inhibitor, apocynin decreases the expression of gp91phox and
In diabetes, NADPH oxidase is a major source of generation of ROS. On the other hand, a major defense of endothelial cells against oxidative stress is nitric oxide (NO), synthesized by NO synthase (NOS). The NO synthase family consists of three isoenzymes: NO synthase-1 (NOS-1 or neuronal NOS, nNOS), NO synthase-2 (NOS-2 or inducible NOS, iNOS), and NO synthase-3 (NOS-3 or endothelial NOS, eNOS). NOS-1 and NOS-3 are constitutively expressed in various cell types, while NOS-2 is induced under conditions of inflammation and tissue injury.

The activation of NOS-1 and NOS-3 leads to the production of NO, which is important for maintaining vascular tone and inhibiting platelet aggregation. In contrast, the activation of NOS-2 results in the production of a burst of NO, which is important for the regulation of immune and inflammatory responses.

In diabetic nephropathy, the activation of NOS-2 is increased in response to high glucose, leading to the production of a large amount of NO. This increase in NO production is associated with the development of diabetic complications.

REFERENCES

PKC-Protein Kinase C, ROS-Reactive oxygen species, eNOS-endothelial nitric oxide synthase, CAM-1 (Intercellular Adhesion Molecule 1), Akt also known as Protein Kinase B (PKB), Tetrahydrobiopterin deficiency (THBD, BH4D) also called THB or BH4D deficiency. High glucose (HG)-induced ROS formation resulted in the phosphorylation of Ser36-p66shcA protein that promoted further ROS formation. ROS overproduction activated Akt/ PKB kinase, which phosphorylated and inactivated FOXO3a protein, enhanced oxidative stress. Diabetes increased the levels of transforming growth factor TGFβ that led to the inactivation of phospho-FOXO3a protein and the enhanced oxidative stress.

CONCLUSION AND DISCUSSION
Present findings demonstrate importance of NADPH mediated ROS and RNS signaling in many enzymatic cascades regulating the development of diabetic nephropathy. It might also be suggested that the deregulation of ROS signaling is probably the beginning of pathological changes in diabetes.

Investigation of ROS and RNS signaling in enzymatic cascades which are responsible for developing diabetic nephropathy could be a fascinating task, promising the discovery of new pharmaceutical agents and methods for the treatment of these pathologies. Aforementioned findings suggest the potential usefulness of the inhibitors of ROS & RNS signaling in gene/enzymatic processes for the treatment of diabetic nephropathy.