INTRODUCTION

Vascular endothelium dysfunction is the hallmark of pathological conditions associated with metabolic disorder, and among these metabolic disorders, diabetes mellitus, hypercholesterolemia, hypertension and hyperhomocysteinemia are the leading cause of morbidity and mortality. The available drugs for treatment of these conditions focus mainly on control of blood sugar levels, cholesterol level or blood pressure. However macro and micro vascular complications of diabetes mellitus, hypercholesterolemia, hypertension and hyperhomocysteinemia are delayed but not prevented or cured, and continuous administration of these drugs is required.

Vascular endothelium plays a vital role in capillary transport of nutrients and drugs and regulates angiogenesis, homeostasis, as well as vascular tone and permeability. As a major regulator of local vascular homeostasis, the endothelium maintains the balance between vasodilatation and vasoconstriction, procoagulant and anticoagulant, prothrombotic and antithrombotic mechanisms by releasing vasoactive mediators viz. endothelium dependent relaxing factor (EDRF), endothelium dependent contracting factor (EDCF), endoperoxides, thromboxanes, endoperoxides, prostanoids, Von Willebrand factor (VWF), factor VIII antigen, endothelin -1, angiotensin II, tissue plasminogen activator (t-PA), thrombomodulin, adenosine nucleotides (ADP and ATP), matrix metalloproteinases and heparin (Tirziu and Simons., 2009).

The cellular and molecular mechanisms underlying pathological conditions of diabetes mellitus (Triggle and Ding., 2010), hypercholesterolemia (Bai et al., 2010), hypertension (Sowers., 2004) and hyperhomocysteinemia (Guilliams., 2004) lead to an imbalance of phosphorylation and dephosphorylation status of lipid and protein kinases that cause modulation of vascular L-arginine /nitric oxide synthetase (eNOS),
to produce vascular endothelium dysfunction. The increased expression of eNOS and hence increased production of nitric oxide, is important for cardiovascular homeostasis, vessel remodeling and angiogenesis (Napoli et al., 2010). The complex regulation of eNOS in cardiovascular physiology occurs at multiple stages. eNOS mRNA levels are controlled both at the transcriptional and post-transcriptional phases, and epigenetic mechanisms appear to modulate tissue-specific eNOS expression. The eNOS enzyme reversibly associates with a diverse family of protein partners that regulate eNOS sub-cellular localization, catalytic function, and biological activity (Dudzinski and Michel., 2007).

Diabetes mellitus causes the activation of aldose reductase, polyol pathway and advanced glycation-end-product formation that collectively affect the phosphorylation status and expression of endothelial nitric oxide synthetase (eNOS) and causes vascular endothelium dysfunction (Hurks et al., 2009). Elevated homocysteine levels have been associated with increase in LDL oxidation, generation of hydrogen peroxides, superoxide anions that increased oxidative degradation of nitric oxide (Osto and Cosentino., 2010). Hyperhomocysteinemia has been reported to increase the endogenous competitive inhibitors of eNOS viz- L-N-monomethyl arginine (L-NMMA) and asymmetric dimethyl arginine (ADMA) that may contribute to vascular endothelial dysfunction (Jakubowski., 2006). Hypercholesterolemia stimulates oxidation of LDL-cholesterol, release of endothelins, and generation of ROS. The increased cholesterol and triglyceride level and decreased protective HDL level, decreases the activity and expression of eNOS and disrupts the integrity of vascular endothelium, due to oxidative stress (Chapman and Andrei., 2008) Hypertension also stimulates release of endothelins, vasoconstrictor prostanoids,
angiotensin II, inflammatory cytokines, xanthine oxidase and, thereby, reduces bioavailability of nitric oxide (Probstfield and O'Brien, 2010).

Phosphatidylinositol-3 kinase is a ubiquitous enzyme involved in plethora of cell signaling including the endothelial cells (Lindmo & Stenmark, 2006) and it has been reported that signaling through this enzyme and its downstream pathway viz Phosphoinositide-dependent kinase (PDK)/ protein kinase B (Akt) and eNOS is impaired in diseased conditions (Liang and Slingerland, 2003). In recent years, increasing evidence indicates phosphoinositide 3-kinases (PI3K) as crucial signal transducing elements that regulate communication across the plasma membrane. PI3K generate lipid secondary messengers that trigger a plethora of intracellular responses ranging from metabolic regulation to cell proliferation, survival, and migration (Trimarco and Crispo, 2007).

PI3K acts on membrane phospholipids and activate three classes of down stream signaling molecules 1) AGC family of S/T protein kinases such as protein kinase A, protein kinase B/Akt i.e. Akt 1, Akt 2, Akt 3, protein kinase G, P70 S6 kinase, P90 ribosomal S6 kinase, atypical protein kinase C, glucocorticoid regulated kinase, phosphoinositide dependent kinase, 2) Guanine nucleotide exchange protein of Rho family of GTPase 3) TEC family of tyrosine kinase. PDK causes phosphorylation and activation of AKT at kinase domain and Akt has been documented to modulate various sub-cellular effectors such as proapoptotic factors: BAD, Caspase 9, forkhead transcription factors, NF K-β, mTOR/P70 S6K, eNOS and translocation of Glut-4 to mediate cell growth and cell survival, protein synthesis and glucose metabolism (Alfonso et al., 2004; Knight et al., 2006)

One important downstream effector of PI3K is the 3-phosphoinositidyl dependent kinase-1 (PDK1) (Lemmon, 2007). PDK1 is a member of the AGC family
of protein kinases and like all other AGC kinases, requires phosphorylation at its own T-loop residue (Ser241) in order to be activated. It possesses the intrinsic ability to phosphorylate its own T-loop residue and thus is constitutively active. PDK-1 plays an important role in the regulation of cell survival, differentiation, and proliferation; it phosphorylates and activates a group of related protein kinases belonging to the AGC family (Alfonso et al., 2004). These include isoforms of Akt, p70 ribosomal S6 kinase (S6K), p90 ribosomal S6 kinase (RSK), PKC, and serum- and glucocorticoid induced protein kinase (SGK). PDK1 regulates cell proliferation and cell cycle progression by controlling the expression of cyclin D1 and p27Kip1. PDK1 plays an essential role in regulating insulin-induced glucose uptake in adipocytes by activation of PKB and S6K as well as recruitment and translocation of GLUT4 glucose transporter to the plasma membrane (Vanhaesebroeck et al., 2001). Recent evidence indicates that PDK1 is essential for the motility of vascular endothelial cells (ECs) and is involved in the regulation of their chemotaxis and migration at time of injury. Thus deregulation of PDK-1 physiological function in pathological conditions of diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia and hypertension may be responsible for vascular endothelium dysfunction.

The serine/threonine protein kinase B (PKB, also known as Akt) constitutes an important node in diverse signaling cascades downstream of growth factor receptor tyrosine kinases (Vanhaesebroeck & Alessi, 2000). Akt plays an essential role in cell survival, growth, migration, proliferation, polarity, and metabolism (lipid and glucose); cell cycle progression; muscle and cardiomyocyte contractility; angiogenesis; and self-renewal of stem cells. A major outcome of Akt activation is toward cell survival and cell growth; many Akt substrates play important roles. Among those substrates are: (1) regulators of cell survival or cell death, such as
Bad, caspase-9, ASK1, apoptosis signal-regulating kinase 1 (ASK1), forkhead box O transcription factors (FoxOs), Bim1, FasL, inhibitor of (TSC1/2), mTOR, elongation-initiation factor 4E binding protein-1 (4E-BP1), and S6K; (4) regulators of angiogenesis, such as mTOR and hypoxia-inducible factor-1 (HIF-1); and (5) regulators of cell metabolism, such as glucose transporter 1 (Glut1), GSK3, and a Ras homologue enriched in brain (RheB). Akt protein can be degraded by the ubiquitin proteasome-dependent pathway, caspase-mediated cleavage, and caspase dependent ubiquitination (Shiojima and Walsh., 2002). Altered Akt activity has been associated with cancer and other disease conditions, such as diabetes mellitus, neurodegenerative diseases, and muscle hypotrophy. Thus dysregulation of Akt may be implicated in vascular endothelium dysfunction.

Protein tyrosine phosphatase (PTPase) regulates cellular processes involved in pathological vessel wall functions (Sugano et al., 2004) and has been reported to inhibit the activity of survival pathway AKT (Shah and Singh., 2007), enhance proapoptotic genes such as Caspase, BIM, BAD, BCL-2 (Ruvolo et al., 2002) and reduce the levels of antioxidant enzymes such as catalase, glutathione peroxidase and super oxide dismutase (Ignarro et al., 1988). PTPase also causes proteolysis of protein component of focal adhesion complexes such as FAKinase, paxillin, P13Fas necessary for endothelial cell survival (Nagata et al., 1997). Moreover, PTPase also decreases number and viability of endothelial progenitor cells and thus reduce postnatal blood vessel repair and enhance the rate of endothelial cell senescence (Hartmut et al., 2001). PTPase causes dephosphorylation of tyrosine kinase, SHIP, insulin receptor, leptin receptor (Topping et al., 2000; Molero et al., 1998), and has been implicated in erectile dysfunction (Ferrini et al., 2007), congestive heart failure (Vercauteren et al., 2006), ischemia reperfusion injury (Sugano et al., 2004).
and neurodegenerative disorders. Thus, Protein tyrosine phosphatases may be implicated as a potential target site in interventions for vascular endothelium dysfunction.

The present study was, therefore, designed to investigate the possible involvement of pathways viz PI3K and its downstream pathway PDK/AKT in the impaired vascular endothelium dysfunction in diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia and hypertension.