CHAPTER 1
INTRODUCTION
Cerebral stroke is a clinical syndrome caused by impairment of cerebral blood flow (CBF) either by occlusion (ischemic) or rupture of blood vessel (hemorrhagic) supplying blood to the brain. Historically, it was called "apoplexy", because the very way it strikes down people. This is due to the initiation of multifaceted and complex cellular and molecular mechanisms responsible for cell survival/damage. It has become the second commonest cause of death (Lavados et al., 2007) and it is presumed that 1/6 of all human beings will suffer at least one stroke in their lives (Seshadri et al., 2006). Furthermore, stroke is the principal cause of adult disability in the world with approximately one third of patients who survive 6 months are dependent on others (Warlow, 1998). The stroke survivors experience loss of vision and/or speech, paralysis, and confusion. Stroke poses huge socioeconomic burden absorbing 6% of all health care budgets and the fact that life expectancy increases globally one can assume that stroke is already, and will continue to be, the most challenging disease (Durukan and Tatlisumak, 2007). Previous incidence of stroke significantly increases risk of further episodes. Certain racial, ethnic and socioeconomic groups are also at the greater risk of stroke.

Stroke has always been associated with various risk factors such as diabetes/hyperglycemia that appears to play an important role in severity of the disease. Diabetes per-se is one of the most serious long-term health problems responsible for pathogenesis and development of many diseases including ischemic cerebrovascular disease and when poorly managed can be severely debilitating, even lethal, and have enormous social and economic consequences (Zimmet et al., 2001). Diabetes affects numerous systems and functions through a myriad of molecular mechanisms (Brownlee, 2001). Patients with diabetes are at least 4-12 times more prone to stroke than non-diabetic subjects and are more likely to suffer with increased morbidity and mortality after stroke. Clinical studies on diabetic patients support the fact that raised plasma glucose concentrations augment vascular, physiological and morphological alterations and contribute to the marked damage. The acute hyperglycemia and chronic diabetes can aggravate brain damage due to transient global or forebrain ischemia (Nedergaard, 1987; Siesjo et al., 1996; Li and Siesjo, 1997; Li et al., 1998; Gisselsson et al., 1999) and ischemic region rapidly develops neuronal necrosis with subsequent possibility of pannecrotic lesions. In animal models, pre-existing short-term hyperglycemia that was not reversed after more extended periods of middle cerebral artery occlusion (MCAo), significantly increased infarct volume compared to
normoglycemic animals (Gisselsson et al., 1999). In more severe or prolonged reperfusion periods, impaired CBF was correlated well with increased infarct volume (Palmon et al., 1995).

The process by which diabetes worsens morphologic outcome is uncertain. However, provoking anaerobic metabolism, lactic acidosis and free radical production, hyperglycemia may exert direct membrane lipid peroxidation and cell lysis in metabolically challenged tissue. Moderately and severely increased blood glucose has been found to further alter the metabolic state and mitochondrial function in the area of ischemic penumbra. The evolution of an infarction is accompanied by glutamate release mediating repeated waves of spreading depression (SD), another mechanism believed to propagate the necrosis of the penumbral tissue. Although hyperglycemia alone did not trigger early-response genes in the cortical tissue of rats, in conjunction with induced SD, the expression of c-fos and cyclooxygenase-2 (COX-2) were substantially increased (Koistinaho et al., 1999). This suggests that elevated glucose may trigger intracellular biochemical cascades also by altering early gene expression in metabolically challenged neurons. The blood brain barrier (BBB) is vulnerable to hyperglycemia, presumably through the liberation of lactic acid and free radicals.

Recent studies have proposed that cerebral ischemia may mediate cellular damage through apoptosis, which otherwise maintain cellular homeostasis by eliminating redundant or damaged cells during development. Moreover, apoptosis is regulated during high glucose level, as in diabetes, via multiple complicated signaling mechanisms (Kowluru, 2005) involving mitochondrial dysfunction as one of the key responses (Brownlee, 2001; Li et al., 2001; Chen and Swanson, 2003; Muranyi et al., 2003). However, inappropriate or excessive apoptosis in any scenario including cerebral ischemia will have negative outcome. It is usually initiated in penumbral region depending upon the availability of energy through the sequence of events involving activation of caspase-3 either through death receptor activated caspase-8 or cytochrome c mediated apoptosome formation. Previous study has documented the role of intrinsic apoptotic pathway in streptozotocin (STZ)-diabetic rats following cerebral ischemia (Ding et al., 2004). Caspase-3 is thought to execute cell death program with specific and extensive apoptotic DNA fragmentation by caspase-activated deoxyribonuclease (CAD) (Liu et al., 1997; Mukae et al., 1998; Porter and Janicke, 1999). However, it is reported that expression of caspase-3 diminishes markedly after birth and remains extremely low in adult rat brain (Ni et al., 1998; Shimohama et al.,
1999; Zhu et al., 2005) raises doubts about its clear involvement in large scale neuronal damage. Further, accumulating evidence indicate the existence of caspase independent mechanisms of neuronal cell death in a condition, where caspases are not activated or caspase inhibitors do not offer neuroprotection in certain neuronal populations in experimental models of stroke (Johnson et al., 1999; Lankiewicz et al., 2000; Rideout and Stefanis, 2001; Zhan et al., 2001). Furthermore, in a number of neuronal cell death paradigms in which caspases does play a role, its inhibition at or downstream of the apoptosome delays, but does not prevent the occurrence of caspase-independent cell death (Miller et al., 1997; Stefanis et al., 1999; D'Mello et al., 2000; Keramaris et al., 2000; Selznick et al., 2000; Johnson et al., 1999; Lankiewicz et al., 2000). Therefore, it is speculated that at least in certain neuronal death paradigms involving massive cellular damage, caspases inhibition or low activation may recruit or activate compensatory cell death processes.

One of the key components of the caspase independent cell death pathway is the flavoprotein apoptosis inducing factor (AIF), a mitochondrial oxidoreductase. AIF is synthesized as a ~67 kDa preprotein (613 amino acid residues) with an N-terminal prodomain containing mitochondrial localization sequences (MLS) and then processed to a ~62 kDa mature protein (Susin et al., 1999; Otera et al., 2005). However, it gets further processed into ~57 kDa form by activated calpains and/or cathepsins (Otera et al., 2005; Polster et al., 2005; Yuste et al., 2005). The role of AIF in neuronal cell death depends on its translocation to nucleus due to C-terminal nuclear localization sequence (NLS) in response to excessive activation of poly(ADP-ribose)polymerase-1 (PARP) after the induction of various types of acute neuronal injury in vitro and in vivo (Yu et al., 2002; Zhang et al., 2002; Cao et al., 2003; Plesnila et al., 2004; Wang et al., 2004; Yu et al., 2006) thereby leading to an intrinsic cell death program different from PARP induced necrosis.

Present study, therefore, is an attempt to reveal the role of mitochondrial dysfunction to downstream cell death activation pathway(s) mediated by caspase-3 and AIF, since it was assumed that caspase-3 activation alone may not be able to culminate into increased cellular damage in diabetes following stroke. Further, the role of reactive oxygen species (ROS) in cellular damage has been analyzed using melatonin, an endogenous pineal gland product with established antioxidant property. The deregulation of molecular stress marker heat shock protein 70 (HSP70) expression was
also studied to assess the relative degree of stress in STZ-diabetic condition following cerebral ischemia. Further studies will be undertaken to analyze the role of caspase-3 and AIF to analyze caspase-dependent and independent apoptotic mechanisms following mitochondrial dysfunction.