CHAPTER 6
SUMMARY
Cerebral stroke is a heterogeneous syndrome caused by multiple mechanisms involving disruption of CBF with subsequent tissue damage. There are several risk factors associated with stroke including diabetes, which increases the incidence particularly of ischemic stroke by at least 4-12 times. Thus, reduction in CBF impairs the neurological function and activates several intricate and overlapping signaling mechanisms, which modulate the cellular survival and thereby imparts severe impact on morbidity and mortality. However, the mechanisms by which diabetes aggravates ischemic brain damage are still not very elusive. So, to reduce potential neurological morbidity, there has to be a sufficient understanding of the pathophysiological mechanisms involved in exaggerating the ischemic brain damage in diabetes. Since, the long term goal in stroke research is based on the idea to reduce neuronal loss and to improve functional recovery after cerebral ischemia-reperfusion. Success in this long-term goal will depend upon the proper understanding of the molecular mechanisms that causes neuronal dysfunction and to identify the main molecular processes that promote effective repair functions in the brain, thereby reduce injury and/or cell death. The present study therefore, is an attempt to unravel the cellular and molecular mechanisms of enhanced stroke damage in diabetes.

The study was conducted on male normoglycemic and streptozotocin (STZ)-diabetic male Sprague-Dawley rats (280±20g). Cerebral ischemia was induced by MCAo for 0.5, 1 and 2 h and 24 h reperfusion for preliminary investigation and to obtain basal data for choosing the appropriate time of I/R for detailed investigation of underlying mechanisms of cerebral damage.

It was observed that I/R injury impaired the brain function, which was clearly depicted by altered sensori-motor functions. The diabetic animals showed significantly higher ND score at each time points of I/R as revealed by varying degree of flexion, contralateral circling, hemiparesis and non-spontaneity. This was clearly supported by increased infarct size in diabetic as compared to normoglycemic rats at respective time points of I/R injury. Further, it was found that ischemic brain damage was largely confined to the striatum at early time point (0.5/24, 1/3, 1/6 h) of I/R injury in diabetic animals but it extends to the cortical region upon increasing the ischemia or reperfusion time. The progressive increase in cerebral damage upon extending the I/R time (2/24 h) resulted in increased mortality in diabetics. The increase in cerebral damage seems to be mediated by necrotic and apoptotic cell death mechanisms as revealed by H&E staining. The cellular damage was greater in striatum following I/R of 1/24 h and
predominantly necrotic in nature with cavitations as an indicator of cell loss, whereas the cortical region displayed cellular damage characterized by apoptotic features. The hippocampus showed normal cellular morphology. There results were further demarcated at nuclear level and it was found that striatum being greatly affected by ischemic insult and displayed DNA fragmentation lacking characteristic morphological features of apoptosis. The large number of neurons surrounding the infarct in cortical area exhibited prominent DNA damage mediated by apoptotic phenomenon. The cell death in these two very regions clearly depicts the severity of ischemic stress.

The reasons for enhanced cerebral damage seems to be oxidative stress induced activation of cell death mechanisms as indicated by higher MDA and reduced GSH level in diabetics.

The systematically carried out detailed investigation for biochemical, cellular, and molecular basis of diabetes induced increased cellular stroke damage at 1 h of ischemia and 3, 6, 12 and 24 h of reperfusion further revealed that cerebral ischemia induces increased early (1/3 h of I/R) initiation of apoptosis in diabetes in cortex and striatum area. The apoptotic events increased further at 1/24 h in cortical area of diabetic. However, the decrease in apoptotic changes in striatal area at 1/24 h in diabetic and normoglycemic animals suggests that the striatal area is severely compromised due to reduction of CBF and thus seems to form the core region. This is further supported by reduced HSP70 expression, which was used as a marker to demarcate core and penumbra. This may also be due to inhibition of cerebral protein synthesis or loss of cells due to large cavitations in striatal area. In line with the increased MDA level in diabetic following I/R injury, the predominance of oxidative stress was further confirmed by increased ROS level in the affected brain nuclei of diabetic rats. Increased ROS generation seems to have direct effect on mitochondria and nucleus since melatonin treatment not only normalized mitochondrial function but also reduced the TUNEL positive cells, which indicates DNA fragmentation. The mitochondrial potential alteration following cerebral ischemia may be due to ROS generation and Bax induced pore formation. The mitochondrial dysfunction indicates the activation of cell death pathway mediated by cytochrome c and AIF release form mitochondria, which was greater in diabetics following I/R injury.

Cytochrome c activates caspase-3 and the activation of caspase-3 was found greater together with the mRNA in the cortical area of diabetic rats. Caspase-3 activation was further supported by cleavage of PARP as early as 3 h post reperfusion.
in diabetics. Thus, significant increase in apoptosis as indexed by TUNEL and caspase-3 localization at 24 h post reperfusion in cells with apoptotic morphology strongly suggest the apoptotic cellular damage under diabetic condition.

Further, some cells in the present study showed peripheral chromatin condensation on the nuclear membrane as revealed by TUNEL and Hoechst nuclear staining. These features are suggestive of cells undergoing caspase-3 independent apoptosis. Thus, nuclear translocation of AIF found in the present study is in direct marker to such cell death phenomenon. The increased expression of PARP as early as 3 h post reperfusion seems to be responsible for propagating the signal for AIF release. However, the transcriptional regulation of AIF seems strictly dependent on the function of AIF in different cellular compartment and severity of stress in ischemic affected brain nuclei, which is supported by the decreased expression of HSP70 in striatum of diabetics. Further, this also have direct correlation with the increased translocation of AIF at respective time point. Similarly expression of HSP70 at mRNA level indicates the inhibition of cerebral protein synthesis in severely damaged striatal area. The contribution of ROS in enhancing cerebral damage in diabetics was clearly demarcated by significant reduction in infarct size and improved neurological function with melatonin when administered at the onset and 6 h post reperfusion.

In conclusion, the results of the present study revealed that diabetes enhances ischemic stroke damage by activation of mitochondria-mediated cell death pathway, through caspase-3 dependent and independent mechanisms. This may partly be due to enhanced ROS induced oxidative stress generation since, melatonin reduced ROS level, normalized mitochondrial potential, reduced DNA damage and infarct size, which may be responsible for improved neurological function. This also indicate that in addition to early activation of damaging cellular signaling, cerebral ischemia activate additional deleterious mechanisms around 6 h of reperfusion which seems to enhance the brain damage in diabetes.

The ultimate benefit of our work will therefore lies in providing detailed cellular and molecular basis of increased brain damage in diabetes, which is expected to help in development of genetic, biochemical, and pharmacological interventions in the management of cerebral stroke particularly in diabetes.