



## The Effect of Diabetes Mellitus on Apoptosis in Hippocampus: Cellular and Molecular Aspects

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### ABSTRACT

**Background:** Diabetes mellitus is associated with cognitive deficits in humans and animals. These deficits are paralleled by neurophysiological and structural changes in brain. In diabetic animals, impairments of spatial learning, memory, and cognition occur in association with distinct changes in hippocampus, a key brain area for many forms of learning and memory and are particularly sensitive to changes in glucose homeostasis. However, the multifactorial pathogenesis of diabetic encephalopathy is not yet completely understood. Apoptosis plays a crucial role in diabetes-induced neuronal loss in hippocampus.

**Methods:** The effects of diabetes on hippocampus and cognitive/behavioral dysfunctions in experimental models of diabetes are reviewed, with a focus on the negative impact on increased neuronal apoptosis and related cellular and molecular mechanisms.

**Results:** Of all articles that were assessed, most of the experimental studies clearly showed that diabetes causes neuronal apoptosis in hippocampus through multiple mechanisms, including oxidative stress, inhibition of caspases, disturbance in expression of apoptosis regulator genes, as well as deficits in mitochondrial function. The balance between pro-apoptotic and anti-apoptotic signaling may determine the neuronal apoptotic outcome *in vitro* and *in vivo* models of experimental diabetes.

**Conclusions:** Dissecting out the mechanisms responsible for diabetes-related changes in the hippocampal cell apoptosis helps improve treatment of impaired cognitive and memory functions in diabetic individuals.

**Keywords:** Apoptosis, central nervous system complication, diabetes mellitus, hippocampus

### INTRODUCTION

Diabetes mellitus is the most common serious metabolic disorder in humans and is clinically defined as a group

of metabolic diseases characterized by hyperglycemia as a result of defects in insulin secretion or resistance to insulin action or both.<sup>[1,2]</sup> There are multiple types of diabetes, but the most common forms are type 1 and type 2 diabetes. Type 1 diabetes (previously defined as insulin-dependent diabetes mellitus) is characterized by an autoimmune-mediated destruction of pancreatic  $\beta$ -cells,

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leading to insulin deficiency. Consequently, patients require insulin treatment for survival and withdrawal from insulin treatment leads to ketoacidosis. Type 2 diabetes (previously defined as noninsulin-dependent diabetes mellitus) is characterized by insulin resistance and relative (rather than absolute) insulin deficiency. Treatments aim to reduce insulin resistance (for example, with diet, exercise or drug therapy) and to increase endogenous insulin secretion. Eventually, exogenous insulin can be needed.<sup>[2-5]</sup> The epidemiology of diabetes mellitus in developed countries has been assessed using screening methods to detect undiagnosed cases. The incidence of type 1 diabetes is 8–20 per 100,000 patient-years in children up to 18 years of age. After the age of 20 years, the incidence is lower with little further effect of age.<sup>[6]</sup> The incidence of type 2 diabetes is estimated to be about 5 per 100,000 patient-years in subjects up to 29 years of age, increasing sharply up to 500 per 100,000 patient-years in subjects over 70 years.<sup>[7]</sup> The prevalence of diabetes ranges from about 2% in subjects aged 20–44 years to 18% in subjects aged 65–74 years, and over 20% in subjects aged 85 years and older.<sup>[8,9]</sup> Impaired glucose tolerance can be present in 7–10% of the subjects of 50 years and older.<sup>[9]</sup> Because the average age of the western population is increasing, and the incidence of type 2 diabetes is particularly high among the elderly, the overall prevalence of diabetes will increase significantly in the next few decades.<sup>[6,7,9]</sup>

## METHODS

The keywords “diabetes,” “Apoptosis,” and “hippocampus” were searched in PubMed, MEDLINE, and EMBASE databases with the following limits: English language and published in the last 15 years (2001–2015). Article titles and abstracts were screened, and potentially relevant articles were retrieved and evaluated. This review included published studies that examined the effect of diabetes on hippocampal apoptosis [Table 1], and it summarizes and synthesizes the highlights of the selected articles. The known cellular and molecular mechanisms involved in hippocampus neuronal apoptosis (i.e., gene regulators, caspases activity, and oxidative stress) are also discussed. Most of the reviewed behavioral studies involved the evaluation of hippocampal apoptosis in a model of experimental diabetes.

## RESULTS

### Diabetic encephalopathy

The evidence of the impact of metabolic diseases such as diabetes mellitus on central nervous system (CNS) considerably grew up in the last decade.<sup>[10-14]</sup> Neuroradiological and neurophysiological

studies provide further evidence that both types of diabetes are associated with functional and structural disturbances in the brain.<sup>[15-19]</sup> The development of these complications is dependent on the duration of diabetes and the quality of metabolic control, and can only be partially prevented by intensive insulin treatment.<sup>[20,21]</sup> These disturbances are related to acute hypoglycemia or severe hyperglycemia and stroke and are referred to as diabetic encephalopathy, a term that encompasses functional impairment of cognition, cerebral signal conduction, neurotransmission and synaptic plasticity, and underlying structural pathology associated with diabetes.<sup>[22,23]</sup> Evidence from several line of studies clearly indicated that individuals with type 1 or type 2 diabetes have been shown to have poorer performance in a wide range of neuropsychological tests.<sup>[24,25]</sup> Type 1 diabetic patients might have impairments in learning and memory, problem-solving, and mental and motor speed. The deficits are generally modest, but can occasionally be severe.<sup>[13,21,26]</sup>

The results of neuropsychological studies of type 2 diabetic patients are also more consistent.<sup>[11,12]</sup> Moderate cognitive impairment is reported, particularly in tasks involving verbal memory or complex information processing. Basic attentional processes, motor reaction time, and short-term memory are relatively unaffected.<sup>[27]</sup> Moreover, recent epidemiological studies demonstrate an association between diabetes and vascular dementia as well as Alzheimer’s disease.<sup>[10,12-16]</sup>

Over recent decades, studies have also provided evidence indicating the deleterious effects of type 1 diabetes mellitus on structure of the brain.<sup>[28,29]</sup> Duration-related or chronic effects of type 1 diabetes mellitus on the brain are manifested at all levels of CNS from microscopic to macroscopic level.<sup>[18,19]</sup> Macroscopically neuroimaging studies have demonstrated a high incidence of abnormalities such as temporal lobe sclerosis, declines in white matter volume in parahippocampal gyrus, temporal and frontal lobes as well as decreased gray matter volumes and densities of the thalami, hippocampi, insular cortex, superior and middle temporal, and frontal gyri.<sup>[19,30]</sup>

In experimental models of type 1 diabetes mellitus, a vast spectrum of neuronal changes has been reported. These pathological abnormalities include synaptic and neuronal alterations, degeneration, increased cerebral microvascular permeability, and neuronal loss which collectively can lead to cognitive impairment and higher risk of development dementia.<sup>[31-33]</sup>

In summary, diabetes is associated with an “encephalopathy” characterized by slowly progressive, clinically prominent cognitive impairments accompanied by brain neurophysiological and structural disorders.

**Table 1: Effects of experimental diabetes models on hippocampal apoptosis**

Model	Associated behavior	Treatments that reversed changes	Reference
STZ		Grape seed extract and Vitamin E	Yonguc <i>et al.</i> <sup>[34]</sup>
STZ	Impaired cognition	Insulin replacement	Ghasemi <i>et al.</i> <sup>[35]</sup>
STZ	Impaired spatial memory	Fish oil	Sun <i>et al.</i> <sup>[36]</sup>
STZ		Natural honey and insulin	Jafari Anarkooli <i>et al.</i> <sup>[37]</sup>
STZ		Exercise	Alipour <i>et al.</i> <sup>[38]</sup>
STZ	Impaired spatial memory	Fish oil	Zhao <i>et al.</i> <sup>[39]</sup>
STZ		Sericin	Chen <i>et al.</i> <sup>[40]</sup>
STZ	Impaired spatial memory	Naokangerhao decoction	Chen <i>et al.</i> <sup>[41]</sup>
STZ		dl-3-n-butylphthalide	Zhang <i>et al.</i> <sup>[42]</sup>
STZ	Impaired cognition		Jafari Anarkooli <i>et al.</i> <sup>[43]</sup>
STZ	Impaired cognition	Tocotrienol	Kuhad <i>et al.</i> <sup>[44]</sup>
STZ		Fish oil n-3 essential fatty acid	Cosar <i>et al.</i> <sup>[45]</sup>
BB/Wor	Impaired cognition	C-peptide and insulin replacement	Sima and Li <sup>[46]</sup>
BB/Wor		C-peptide replacement	Li <i>et al.</i> <sup>[47]</sup>

STZ=Streptozotocin, BB/Wor=The BB/Wor rat spontaneously develops a diabetic syndrome similar to that of type I diabetic humans

A better understanding of the underlying mechanisms may allow us to challenge the concept that the accelerated cognitive decline in these individuals is an irrevocable process.

### Cognitive deficits in diabetic rodents

Behavioral studies in diabetic rodents have produced very different results, because of differences in the animal models used and in the duration of diabetes.<sup>[13,48,49]</sup> An important factor appears to be the nature of the stimulus used in the behavioral paradigm.<sup>[48,49]</sup>

The performance of streptozotocin (STZ)-diabetic mice is clearly impaired in relatively simple tasks such as active avoidance in a shuttle box or T-maze.<sup>[50]</sup> The cognitive performance of STZ-diabetic rats has also been tested in the Morris water maze.<sup>[35,36,39,48,51]</sup> Spatial learning in this task involves multiple cognitive components such as problem-solving, enhanced selective attention, formation of internal representations of the external world, and storage and retrieval of relevant information.<sup>[36,39,49-51]</sup>

Young-adult STZ-diabetic rats begin to display learning deficits in the Morris maze ten weeks after the induction of diabetes.<sup>[48,52,53]</sup> In the spatial version of the task, it is indicated that diabetic rats have a poorer comprehension of the task than do controls. While in aged rats (24 months), the diabetic deficit in water-maze performance was larger than expected from the effect in young-adult rats, which suggests that there is an interaction between aging and diabetic cerebral dysfunction.<sup>[52]</sup>

### Effects of diabetes on hippocampus

Numerous studies have shown that experimental diabetes has negative impacts and induce apoptosis in hippocampal neurons via multiple known mechanisms [Table 2]. Hippocampus is one of the most sensitive regions of the brain to the metabolic disorders including diabetes mellitus.<sup>[54,55]</sup> The hippocampus is a

horseshoe-shaped paired structure, with one hippocampus located in the left brain hemisphere and the other in the right hemisphere.<sup>[56,57]</sup> It is a crucial part of the limbic system, which plays a pivotal role in memory formation, emotional, adaptive, and reproductive behaviors<sup>[56,57]</sup> and also is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories.<sup>[58,59]</sup> The hippocampus acts as a memory indicator by sending memories out to the appropriate part of the cerebral hemisphere for long-term storage and retrieving them when necessary.<sup>[58-60]</sup>

The hippocampus itself is divided into two interlocking sectors, the dentate gyrus and the hippocampus proper (cornu ammonis). The dentate gyrus has three layers such as (1) the granular layer containing the densely packed cell bodies of the granule-cells, (2) the molecular layer formed by the intertwining apical dendrites of the granule-cells and their afferents, and (3) the polymorph layer in the hilus of the dentate gyrus containing the initial segments of the granule-cell axons as they gather to form the glutamergic mossy fiber bundle.<sup>[57,59]</sup> Hippocampus proper as an archeocortical structure has been divided into several layers as follows: (1) The alveus; containing the axons of the pyramidal cells, (2) the stratum oriens, a layer between the alveus and the pyramidal cell bodies which contains the basal dendrites of the pyramidal cells, (3) the stratum pyramidale, (4) the stratum radiatum, and (5) the stratum lacunosum/molecular, which are the proximal and distal segments of the apical dendritic tree, respectively. In the CA3 field, an additional layer is recognized in the stratum lucidum, interposed between the pyramidal cell bodies and the stratum radiatum, receiving the mossy-fibers input from the dentate granule-cells. Each CA3 giant pyramidal neuron with large dendritic spines receive as many as 10–50 mossy fibers from dentate gyrus, and send

**Table 2: Effects of experimental diabetes models and measurement methods of hippocampal apoptosis**

Model	Apoptosis measurement methods	Findings	Reference
STZ	TUNEL assay Oxidative stress Bcl-2, Bcl-XL, Bax, caspase-3, -9, -8, Cyt-c, TNF- $\alpha$ , and NF- $\kappa$ B gene expressions	TUNEL positive neurons (increased) Oxidative stress index (increased) Bcl-2 and Bcl-XL gene expression (decreased) Bax, caspase-3, -9, and -8, Cyt-c, TNF- $\alpha$ , and NF- $\kappa$ B gene expressions (increased)	Yonguc <i>et al.</i> <sup>[34]</sup>
STZ	Oxidative stress Phosphorylation of Akt and GSK-3 $\beta$ and proapoptotic genes expression	Oxidative stress index (increased) Akt phosphorylation (increased) GSK-3 $\beta$ phosphorylation (decreased) Pro-apoptotic genes expression (decreased)	Sun <i>et al.</i> <sup>[36]</sup>
STZ	TUNEL assay	TUNEL positive neurons (increased)	Jafari Anarkooli <i>et al.</i> <sup>[37]</sup>
STZ	Bax and caspase-3 genes expression	Bax and caspase-3 genes expression (increased)	Zhao <i>et al.</i> <sup>[39]</sup>
STZ	TUNEL assay Phosphorylated Akt, NF- $\kappa$ B and Bad protein and mRNA expressions	TUNEL positive neurons (increased) P-Akt and NF- $\kappa$ B protein and mRNA expressions (decreased) Bad protein and mRNA expressions (increased)	Chen <i>et al.</i> <sup>[40]</sup>
STZ	Caspase-3, Bax and Bcl-2 proteins expression	Bcl-2 protein expression (decreased) Caspase-3 and Bax proteins expression (increased)	Chen <i>et al.</i> <sup>[41]</sup>
STZ	Caspase-3 protein expression	Caspase-3 protein expression (increased)	Zhang <i>et al.</i> <sup>[42]</sup>
STZ	Bcl-2, Bcl-xL, and Bax mRNA and proteins expressions and Caspases-3 activity	Bcl-2 and Bcl-xL mRNA and protein (decreased) Bax mRNA and protein (increased) Expressions Caspase-3 activity (increased) Bax/Bcl-2 and Bax/Bcl-xL ratios (increased)	Jafari Anarkooli <i>et al.</i> <sup>[43]</sup>
STZ	Quantification of acetylcholinesterase activity, oxidative-nitrosative stress, TNF- $\alpha$ , IL-1 $\beta$ , NF- $\kappa$ B and caspase-3	Acetylcholinesterase activity (increased) Oxidative-nitrosative stress (increased) TNF-alpha expression (increased) IL-1 $\beta$ expression (increased) NF- $\kappa$ B expression (increased) Caspase-3 expression (increased)	Kuhad <i>et al.</i> <sup>[44]</sup>
STZ	Measurement of MDA level SOD and CAT activity Count of apoptotic neurons	MDA level and SOD and CAT activities (increased) Number of apoptotic neurons (increased)	Cosar <i>et al.</i> <sup>[45]</sup>
STZ	TUNEL assay Caspase-3 protein expression	TUNEL positive neurons (increased) Caspase-3 protein expression (increased)	Kang <i>et al.</i> <sup>[61]</sup>
BB/Wor	Oxidative stress Bax mRNA expression Caspase 3 activity Count of caspase 3-positive neurons	Oxidative stress (increased) Expression of Bax (increased) Caspase 3 activity (increased) Number of caspase 3-positive neurons (increased)	Sima and Li <sup>[46]</sup>
BB/Wor	Assessment of DNA fragmentation	DNA laddering (increased)	Li <i>et al.</i> <sup>[47]</sup>

STZ=Streptozotocin, TUNEL=Terminal deoxynucleotidyl transferase dUTP nick end labeling, BB/Wor=The BB/Wor rat spontaneously develops a diabetic syndrome similar to that of type I diabetic humans, GSK-3 $\beta$ =Glycogen synthase kinase-3 beta, TNF- $\alpha$ =Tumor necrosis factor-alpha, IL-1 $\beta$ =Interleukin-1 beta, NF- $\kappa$ B=Nuclear factor-kappaB, MD=Malondialdehyde, SOD=Superoxide dismutase, CAT=Catalase

their axons into the fimbria. New memory formation and consolidation process of events by hippocampus depend on the integrity of hippocampus internal circuits.<sup>[58,59]</sup>

Hippocampus structural complexity has made it vulnerable to the many pathological conditions such as diabetes mellitus.<sup>[13,48,54]</sup> Studies have shown that cell proliferation continues in granular layer of dentate gyrus constantly. This unique neuronal renew is necessary for memory formation.<sup>[62-64]</sup> Any factor disturbing the balance between neuronal proliferations/death may result in memory and learning impairment.<sup>[65,66]</sup> Studies have demonstrated that experimental diabetes causes

decreased granular cells proliferation and neuronal death (necrosis/apoptosis) in CA3 and dentate gyrus regions.<sup>[67-70]</sup> In this line, Li *et al.*, reported hippocampal neuronal death in a spontaneous rat model of type I diabetes mellitus, accompanied by some functional cognitive alterations after a long period of diabetes (8 months).<sup>[69]</sup>

Although neuronal death has been considered the main leading cause of diabetic CNS and peripheral neuropathies, the mode of neuronal death in type I diabetes mellitus has remained as a matter of controversy.<sup>[69,71,72]</sup>

## DISCUSSION

### Effects of diabetes on neuronal apoptosis

Apoptosis, a morphologically distinct form of programmed cell death, is an active, tightly regulated, metabolic, and genetically encoded form of cell death, in which any harm done to the organism by this process is minimized.<sup>[73-75]</sup> It may occur during normal physiological conditions, for example, during embryonic development, where unnecessary cells may die by apoptosis and deregulation of apoptosis may cause pathological conditions such as neurodegenerative diseases.<sup>[76-78]</sup>

This suicidal pathway is characterized by several morphological and biochemical aspects such as the mitochondrial depolarization and alterations in phospholipid asymmetry, membrane blebbing, the condensation of the nucleus and cytoplasm, and the activation of an endonucleolytic process which degrades nuclear deoxyribonucleic acid (DNA).<sup>[78,79]</sup>

The decision to undergo apoptosis may be determined by the balance between pro-apoptotic and anti-apoptotic signaling events triggered by environmental (extracellular) factors, such as tumor necrosis factor- $\alpha$ , Fas ligand (FasL/CD95L), transforming growth factor- $\beta$ , and cytokines. Most growth factors and cytokines promote cell survival, growth, and differentiation by triggering anti-apoptotic signaling on their target cells.<sup>[73,76,80]</sup>

In the previous work, Jakobsen *et al.* showed a significant loss of neocortical neurons in STZ-induced diabetic rats.<sup>[81]</sup> Neuronal apoptosis also occurs in the hypothalamic nuclei in the diabetic Chinese hamster.<sup>[82]</sup> The nature of neuronal apoptosis was not defined in these early studies or it was determined as to whether neuronal loss was associated with cognitive impairments.

Preclinical literature consistently reports that the hippocampal environment of diabetic animals favors apoptosis, as evidenced by significant elevations in apoptotic markers [Tables 1 and 2]. In the type 1 diabetic rat, neuronal counts of hippocampal pyramidal cells were performed in the hippocampal sub-regions (CA1-CA4) and found no significant differences at 2 months of diabetes. Nevertheless, there was 37% and 24% loss of pyramidal cells in the CA1 and CA2 regions, respectively, in 8 months diabetic animals, and no significant decreases were reported in neuronal densities of other hippocampal sub-regions.<sup>[69]</sup> These data suggest a duration-related effects of diabetes on neuronal density decline in hippocampus. There are also several *in vitro* and *in vivo* studies indicating that hippocampal neuronal loss occurs in diabetic animals and this may be a major contributing mechanism to memory and learning impairments.<sup>[31,69]</sup> There is some evidence demonstrating no apoptosis in hippocampal pyramidal neurons or any cognitive deficits in 8 weeks diabetic rats.<sup>[55]</sup> On the other hand,

in 8 months diabetic rats, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeled positive neurons, positive DNA laddering, increased Bax expression, and caspase 3 activity were evident and related to decreased neuronal density and impaired Morris water maze performances [Tables 1 and 2].<sup>[34,37,39,41,43,46,69]</sup> Therefore, duration-related apoptosis is likely to account for the neuronal loss and the concomitant emergence of cognitive impairments in the diabetic animals.

### Diabetes and caspases activity

Many of the morphological changes associated with apoptosis are orchestrated by activation of a cascade of proteases termed caspases. The caspases are a family of cysteine proteases comprising at least 14 members. Activation of caspases represents a point of irreversibility in the cell death process. The regulation of caspases occurs by two distinct molecular signaling pathways, depending on whether the cell signals activating apoptosis originate extracellularly, thereby activating the extrinsic pathway or intracellularly,<sup>[73,83]</sup> thereby activating the intrinsic pathway.<sup>[73,83,84]</sup> There is evidence demonstrating that the diabetes experimental model had hippocampal apoptosis resulting from caspase-dependent mechanisms [Table 2].<sup>[34,37,39,41,43,44,46,61]</sup> Jafari Anarkooli *et al.* found a significant increase in activity of caspases-3, the most important member of caspases family in hippocampus of STZ-induced diabetic rats after 8 weeks.<sup>[43]</sup>

### Diabetes and apoptotic gene regulators

A large number of genes and proteins have been implicated in the control of apoptosis. These can be categorized by their activities at discrete steps in the apoptotic pathway as well as their relationships with specific disease states. A diverse assortment of triggers activates the cascade, which is subject to tight homeostatic regulation by a number of regulators or modulators of the death pathway. The “point of no return” in apoptosis is reached when caspases become enzymatically active in cleaving target proteins (the “executioners” of apoptosis). The Bcl-2 family of factors regulates caspase activation either negatively (e.g. Bcl-2 itself) or positively (e.g. Bax). Other apoptosis modulators reside further upstream and are thought to activate cascades, which are in turn subject to regulation by downstream factors such as Bcl-2.<sup>[75,78,80]</sup>

A variety of factors has also been demonstrated to antagonize apoptotic pathways, both during physiological events (such as normal development) and in pathological states. Heavily studied anti-apoptotic genes in mammalian cells are Bcl-2 and Bcl-XL. Bcl-2 was first identified as part of a common translocation in human follicular lymphoma.<sup>[73,78]</sup> This gene displayed the unusual property of extending cell survival rather than promoting cell proliferation *per se*. Bcl-2 is capable of dimerizing with a number of related factors that comprise a family

of apoptotic regulators. Although their mechanisms of action are not well-understood, some Bcl-2 family members actually promote apoptosis while others (like Bcl-2 itself) promote antagonize apoptosis.<sup>[73,78,83]</sup>

The members of the Bcl-2 family of proteins are important components of the intrinsic apoptotic pathway, regulating mitochondrial outer membrane permeabilization and the release of pro-apoptotic factors, such as cytochrome C, from mitochondria.<sup>[73,78]</sup> In addition, Bcl-2 family members are key regulators of apoptosis because they connect the extrinsic and intrinsic pathways. Bcl-2 was shown to promote tumorigenesis by inhibiting apoptosis, instead of promoting cell proliferation.<sup>[73,78,83,85]</sup>

Members of the Bcl-2 family of proteins are important regulators that facilitate or prevent the release of cytochrome C from mitochondria into the cytoplasm.<sup>[73,78,83]</sup> Bcl-X is a member of the Bcl-2 family. The Bcl-X gene closely resembles Bcl-2 and functions to regulate cell death. This gene acts to inhibit apoptosis similarly to Bcl-2 and antagonizes apoptosis in a variety of tissues.<sup>[73,78,83]</sup> Bax is another pro-apoptotic member of the Bcl-2 family. This and other pro-apoptotic Bcl-2 family members may counteract their ability to protect cell death.<sup>[73,78,83]</sup>

The results of a study by Jafari Anarkooli *et al.* showed that after 8 weeks of diabetes-induction using STZ (60 mg/kg) as diabetogenic agent, Bax expression was considerably increased in hippocampus of STZ-induced diabetic rats at both mRNA and protein levels, while the expression of Bcl-2 and Bcl-xL was significantly reduced at both mRNA and protein levels. They also reported that the Bax/Bcl-2 and Bax/Bcl-xL ratios (as the main index of apoptotic cell death) were significantly increased; signifying hyperglycemia-induced apoptosis in hippocampus of STZ-induced diabetic rats could be mediated by the mitochondrial pathway [Table 2].<sup>[34,39,41,43,44,46,69]</sup>

### Diabetes and oxidative stress

Increasing evidence in both clinical and experimental studies suggests that oxidative stress plays a pivotal role in the pathogenesis of both types of diabetes mellitus [Table 2].<sup>[34,36,44-46,86-88]</sup> High glucose level can stimulate free radical production via glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. On the other hand, weak defense system of the body becomes unable to counteract the enhanced reactive oxygen species (ROS) generation and as a result condition of imbalance between ROS and their protection occurs which leads to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance.<sup>[86-89]</sup> Furthermore, STZ-treated rats that received supplement with antioxidant properties such as fish oil, Vitamin E, and a grape seed extract also had a significant decrease in the number of neurons with apoptotic markers [Tables 1 and 2].<sup>[34,36,39]</sup>

Recent studies have demonstrated free radicals and ROS as the main driving causes of neuronal apoptosis in diabetic paradigm.<sup>[90-92]</sup> Investigations have suggested that ROS and the resulting oxidative stress play a pivotal role in apoptosis. ROS are constantly generated under normal conditions as a consequence of aerobic metabolism.<sup>[91,93]</sup> ROS include free radicals such as the superoxide anion (O<sub>2</sub><sup>-</sup>), hydroxyl radicals, and the nonradical hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). They are particularly transient species due to their high chemical reactivity and can react with DNA, proteins, carbohydrates, and lipids in a destructive manner.<sup>[91,93]</sup> The cell is endowed with an extensive antioxidant defense system to combat ROS, either directly by interception or indirectly through reversal of oxidative damage. When ROS overcome the defense systems of the cell and redox homeostasis is altered, the result is oxidative stress.<sup>[91-93]</sup>

Increasing evidence provides supporting that oxidative stress and apoptosis are closely linked physiological phenomena and are implicated in pathophysiology of some of the chronic diseases including diabetes mellitus, AIDS, amyotrophic lateral sclerosis, retinal degenerative disorders, cancer, Alzheimer's and Parkinson's, and ischemia of heart and brain.<sup>[94-96]</sup>

Oxidative stress is implicated in the pathogenesis of several diseases including diabetes.<sup>[90,91,93]</sup> Evidently, the brain is particularly vulnerable. This is not surprising as the CNS is highly aerobic and thus extremely susceptible to oxidative stress. In addition, the antioxidant defense of the brain is relatively low having almost no catalase and very low levels of glutathione.<sup>[71,72,92]</sup>

Bcl-2 protein has also been shown to prevent cells from apoptosis apparently by an antioxidative mechanism. Taken together ROS, and the resulting cellular redox change, can be part of signal transduction pathway during apoptosis.<sup>[76,78,90]</sup>

It is now established that mitochondria play a prominent role in the regulation of apoptosis. During mitochondrial dysfunction, several essential players of apoptosis, including pro-caspases, cytochrome C, Smac/DIABLO, apoptosis-inducing factor, and apoptotic protease-activating factor-1 (APAF-1), are released into the cytosol following the formation of a pore in the mitochondrial membrane called the "Permeability transition pore." These pores are thought to form through the action of the pro-apoptotic members of the Bcl-2 family of proteins, which in turn are activated by apoptotic signals such as cell stress, free radical damage, or growth factor deprivation. The multimeric complex formation of cytochrome C, APAF-1, and caspase 9 activates downstream caspases leading to apoptotic cell death. All the three functional phases of apoptosis are under the influence of regulatory controls. Mitochondria

also play an important role in amplifying the apoptotic signaling from the death receptors, with receptor recruited caspase 8 activating the pro-apoptotic Bcl-2 protein.<sup>[73,78,80,83,85]</sup>

## CONCLUSIONS

It is now well-established that diabetes mellitus is associated with impaired cognitive function in human and animals. Animal models of both type 1 and 2 diabetes mellitus have confirmed the detrimental effect of chronic high blood glucose levels on learning and memory. Experimentally, learning and memory deficits in STZ-diabetic rats have been associated with neuronal loss, apoptosis induction in neurons of hippocampus. In addition, recent studies revealed the dark neuron production in hippocampus of diabetic rats. Apoptosis is an active, tightly regulated, metabolic, and genetically encoded form of cell death, in which any harm done to the organism by this process is minimized. It may occur during normal physiological or pathological conditions such as diabetes mellitus. Apoptosis is well-regulated by several internal and external regulators such as expression of a number of (pro-apoptotic and anti-apoptotic) genes.

Dissecting out the mechanisms responsible for the induction of apoptosis in hippocampal neurons that partially reflects the effects of diabetes on learning and memory impairments observed in diabetic human and animals helps to improve the therapy. The development of diabetes complications is directly dependent on the duration of the disease and the quality of metabolic control. Until now, it can only be partially prevented by intensive insulin treatment.

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## Conflicts of interest

There are no conflicts of interest.

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