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Review article

The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers

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ABSTRACT

Alzheimer's disease (AD) is neurodegenerative disorder that is associated with memory and cognitive decline in the older adults. Scopolamine is commonly used as a behavioral model in studying cognitive disorders including AD. Many studies have also concurrently examined the neurochemical mechanisms underlying the behavioral modifications by scopolamine treatment. Nonetheless, the scopolamine model has not become a standard tool in the early assessment of drugs. Furthermore, the use of scopolamine as a pharmacological model to study AD remains debatable. This report reviews the scopolamine-induced cellular and molecular changes and discusses how these changes relate to AD pathogenesis.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and is the most prevalent type of dementia affecting the elderly. In AD patients, cholinergic activities are impaired. Cholinergic activities play an essential role in memory and cognitive function. The cholinergic hypothesis was the first among a few theories presented to explain AD pathogenesis [1,2]. This theory proposed that degeneration of cholinergic neurons in the brain contributed substantially to the cognitive decline as seen in AD patients. Now, it is well documented that impairment of cholinergic neuronal circuits play a prominent role in the cognitive deficits associated with aging and neurodegenerative diseases such as AD [3]. The cholinergic hypothesis has then led to the development of cholinergic agonists and acetylcholinesterase (AChE) inhibitors to treat memory and cognitive impairment in mild and moderate AD [3]. The importance of cholinergic function to memory and cognition was first demonstrated by Deutsch [4] over 40 years ago using anti-muscarinic agents such as scopolamine.

Scopolamine is a widely used model to study dementing-related illnesses since it can induce memory and cognitive deficits. This compound was classically employed to antagonize muscarinic acetylcholine (ACh) receptors involved in working memory [5]. Working memory is needed for the performance of complex cognitive-related activities such as reasoning, comprehension and learning [6]. Working memory deficits are a known feature of AD [7,8]. Scopolamine has been shown in many neurobehavioral studies that it can impair memory function in humans and rodents, particularly the short-term memory and learning acquisition [9]. Some of the classical methods for evaluating theses cognitive processes in rodents are the passive avoidance test, water maze test and radial arm maze test [10–14]. Scopolamine has been used in numerous studies to identify and characterize therapeutic targets for AD. Although scopolamine-induced amnesia is an excellent behavioral model to study dementia-related disorders such as AD, the suitability of scopolamine as a pharmacological model to study the cellular and molecular changes that are related to AD remains unclear. The aim of this report is to review the scopolamine-induced alterations in both cellular and molecular levels. Fig. 1 depicts the pathological alterations associated with scopolamine exposure.

2. Scopolamine and the pathological features of AD

Brain atrophy, together with the accumulation of senile plaques and the presence of neurofibrillary tangles, are the pathological characteristics of AD [15,16]. Senile plaques are mainly consists of amyloid-β (Aβ) protein, whereas neurofibrillary tangles are intra-neuronal structures composed of a highly-phosphorylated form of tau protein. Aβ is derived from it precursor protein APP through the sequential proteolytic cleavages by β- and γ-secretases [17], and can be degraded by neprilysin [18]. Scopolamine can cause atrophy and degeneration of brain neurons in rats [19]. Furthermore, it also induces Aβ

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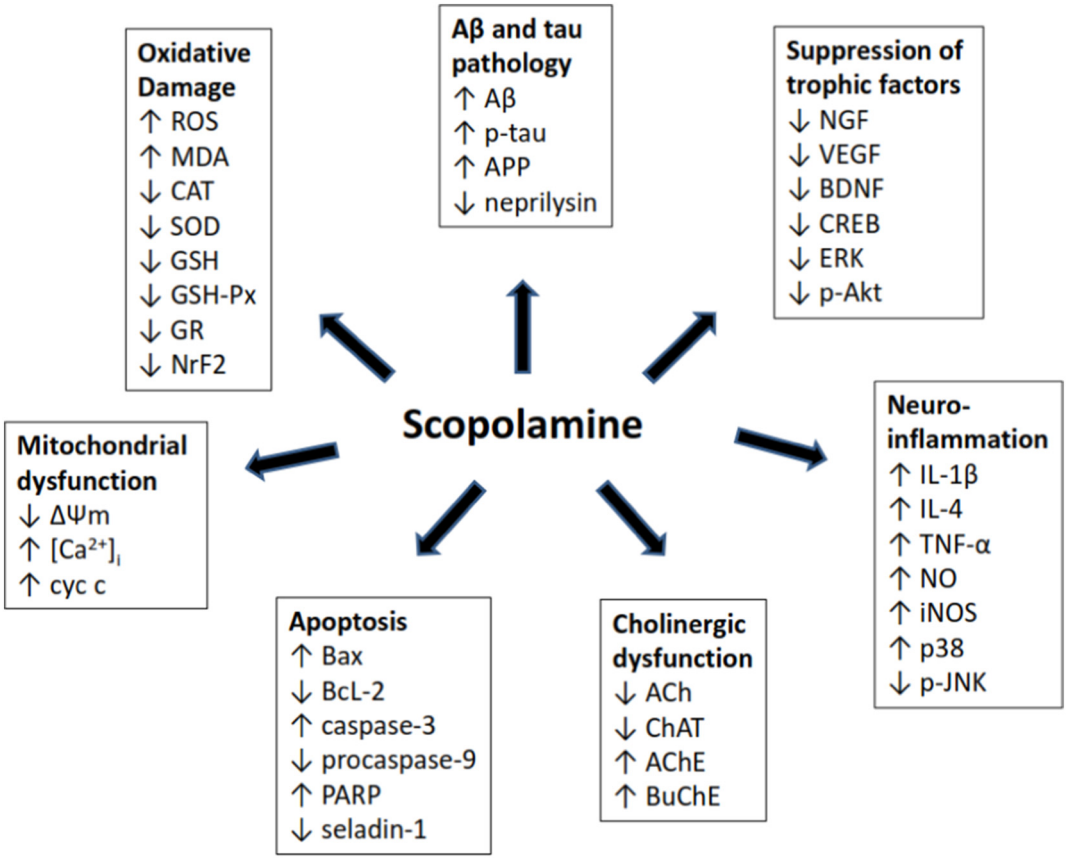

Fig. 1. The cellular and molecular parameter changes associated with scopolamine treatment. AChE, acetylcholinesterase; APP, amyloid precursor protein; BNDF, brain derived neurotrophic factor; BuChE, butyrylcholinesterase; CAT, catalase; ChAT, choline acetyltransferase; CREB, cAMP response element binding protein; cyc c, cytochrome *c*; ERK, extracellular signal-regulated protein kinase; GR, glutathione reductase; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; IL, interleukin; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NGF, nerve growth factor; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; p-Akt, phosphorylated Akt; p-tau, phosphorylated tau; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; ΔΨm, mitochondrial membrane potential.

accumulation in the brain. For instance, administration of scopolamine (2 mg/kg/day, i.p.) for 6 weeks in male Wistar rats increased Aβ protein levels (2.4-fold) and APP mRNA expression (2.7-fold), while suppressing neprilysin mRNA (0.35-fold) in comparison to the control [19]. Scopolamine increased phosphorylated tau protein level by > 2-fold [19,20], but suppressed the gene expression of tau [21]. These findings further support the importance of posttranslational phosphorylation modification in exacerbating tau pathology. Scopolamine also promotes the activity of a tau kinase, known as microtubule affinity-regulating kinase 2, which enhances tau hyper-phosphorylation [22,23]. Scopolamine also caused up-regulation of glycogen synthase kinase 3β mRNA and protein expressions by 2.2 and 5.7 folds, respectively [19]. Glycogen synthase kinase 3β, a tau kinase, may also involve in AD pathogenesis via up-regulation of Aβ production and suppression of acetylcholine synthesis [24].

3. Cholinergic dysfunction

Disruption of cholinergic circuitry has been well documented in several neurodegenerative disorders including AD. Deterioration of central cholinergic neurons impairs cognition, while enhancement of cholinergic signaling improves memory processes [25]. Scopolamine treatment at the concentration of 2 mg/kg (i.p.) reduced the number of AChE-reactive neurons to ~80% of the control at the hippocampus of male Sprague-Dawley rats [26]. Administration of scopolamine at 1 mg/kg (i.p.) also induced a significant decrease in the cholinergic neurotransmitter ACh level to one-third of the control value in brain homogenates from male BAlb/c mice [27]. In addition, scopolamine also decreased ACh level to < 74% in the brain of male Wistar rats [19,28]. ACh is synthesized by choline acetyltransferase and its action is terminated by AChE and butyrylcholinesterase [29]. Scopolamine caused a significant increase in AChE [10,19,20,27,30–36] and butyrylcholinesterase [32] activities. Furthermore, scopolamine also decreased choline acetyltransferase activity [33] and protein expression level [35].

4. Oxidative damage

Another characteristic of AD is elevated oxidative stress in brain neurons [37]. Scopolamine-induced cognitive impairment in animal model is associated with the altered status of oxidative stress in the brain. Lipid peroxidation is the oxidative degradation of lipids where oxidants such as free radicals attack lipids containing carbon-carbon double bond(s) [38]. Malondialdehyde (MDA) is an organic compound generated from lipid peroxidation, and is a bio-marker for oxidative stress [39]. It has been shown that scopolamine-induced cognitive decline was related to drastic elevation of MDA levels in mice [12,27,30,32,40] and rats [20,31,41–44].

Furthermore, scopolamine can impair the cellular antioxidant defense mechanism. Catalase, glutathione peroxidase (GSH-Px), glutathione reductase and superoxide dismutase (SOD) are anti-oxidant enzymes, and reduced glutathione (GSH) is an antioxidant substrate [45,46]. A long-term intraperitoneal injection of scopolamine (3 mg/kg/day) for 14 days induced a significant decrease in SOD level by

≥50% in brain tissues of NIH mice [30,34]. An independent study on male Kunming mice reported that scopolamine administration at the dose of 2 mg/kg (i.p.) decreased SOD activity to 63%, and GSH level by half, in comparison to the vehicle control [40]. In another study, scopolamine treatment (1 mg/kg, i.p.) induced a significant reduction of GSH, GSH-Px and SOD levels to 25%, 60% and 40% of the control value respectively, in male BALB/c mouse brain homogenates [27]. Subcutaneous administration of scopolamine (1 mg/kg) also triggered a significant reduction in SOD, glutathione reductase and GSH-Px activities as well as GSH content in the cortex and hippocampus of ICR mice [47].

A study on male Wister rats by Fan et al. reported that treatment with scopolamine at 1.5 mg/kg inhibited SOD, GSH-Px, and ATPase in the cerebral cortex and hippocampus [41]. Scopolamine decreased the SOD, GSH-Px activity and GSH release in the hippocampus of male Sprague-Dawley rats [31]. Scopolamine administration at the concentration of 1 mg/kg/day for 21 days decreased GSH levels and GSH-Px activities in brain and red blood cells of female Wistar albino rats [13]. Scopolamine (1 mg/kg, i.p.) administered half-an-hour prior to the start of behavioral tests also reduced the levels and activities of GSH, GSH-Px and SOD in the hippocampus of male Wistar rats [44]. Scopolamine also reduced SOD, GSH-Px, and catalase activities, and total GSH level in rat temporal lobe homogenates [43]. An independent study by Ajami et al. who demonstrated that intracranial injection of scopolamine (2 μg) directly into the hippocampal region of male Wistar rats decreased SOD and catalase activities [42]. An in vitro study showed that incubation with scopolamine (3 mM) for an hour also down-regulated the expression of both SOD and GSH-Px to ~80%, and catalase to ~35%, with respect to the control, in C6 rat glioma cells [48].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that activates endogenous antioxidant defense pathways and the production of antioxidant enzymes [49–51]. Scopolamine incubation at the concentration of 5 mM reduced the protein expression level of Nrf2 by > 30% in C6 glioma cells [48]. An animal study on male Wistar rats showed that treatment with scopolamine at 2 mg/kg/day for six consecutive weeks down-regulated the hippocampal transcription factor Nrf2 to ~30% [19,48].

Suppression of antioxidant enzyme activities may lead to high levels of free radicals accumulation in the cell such as reactive oxygen species (ROS). Indeed, scopolamine also induces ROS accumulation. For instance, upon scopolamine treatment, ROS levels were up by ~1.2% and ~1.8% compared to control group in SH-SY5Y [35] and C6 cells [48], respectively. An animal study on male Kunming mice demonstrated that administration of scopolamine at 3 mg/kg (i.p.) increased ROS levels by > 1.5-fold in the cortex and hippocampus [12]. ROS levels were also higher in scopolamine-treated rats [52]. A different study reported that ATPase activity which is sensitive to ROS accumulation was also suppressed by scopolamine treatment [41]. ROS accumulation can lead to apoptosis and mitochondrial dysfunction.

5. Mitochondrial dysfunction

Although mitochondrial dysfunction is involved in AD pathogenesis [53], its role in cognitive impairment induced by scopolamine is not well characterized. However, in the last couple of years, there are a few studies investigating the role of scopolamine in causing mitochondrial dysfunction.

Mitochondrial membrane depolarization, as indicated by JC-1 dye, was significantly higher by ~1.7-fold in scopolamine-treated rats than in the control [52]. The depolarization disrupts mitochondrial membrane potential (ΔΨm) that is vital for maintaining the functionality of the electron transport chain found in the inner mitochondrial membrane to generate ATP during oxidative phosphorylation [54]. Scopolamine also caused loss of ΔΨm in C6 cells [48]. The loss of ΔΨm has been widely due to opening of mitochondrial permeability transition pore, a large conductance pore that spans both inner and out mitochondrial membrane [55]. Once the potential is collapsed, the cells are committed to die due to energy depletion. The loss of ΔΨm is dependent on mitochondrial calcium uptake, and usually followed by a rise in intracellular calcium concentration and an increase in mitochondrial volume [56]. In fact, scopolamine treatment (1 mg/kg/day) for 3 weeks increased cytosolic calcium level significantly by ~1.3-fold in comparison to the untreated control in rat hippocampal and DRG neurons [52]. Nevertheless, whether the loss of ΔΨm occurs as an initiator or effector of apoptosis remains to be elucidated [57].

Wong-Guerra et al. showed that brain mitochondria isolated from scopolamine-treated rats were susceptible to swelling, membrane potential dissipation, calcium efflux and ROS production [58]. On the contrary, in vitro treatment of the brain mitochondria with scopolamine (1–100 μM) did not interfere with the above-mentioned mitochondrial parameters, indicating that the compound does not directly affect the function of the organelle [58].

A gene expression study utilizing cDNA microarray technology by Hsieh et al. showed over-expression of glutamine synthetase, reduction of arginase, and induction of cytochrome *c* in hippocampus when the rats were subjected to scopolamine at 10 mg/kg intracisternally [21]. Glutamine synthetase and arginase are mitochondrial enzymes that are found in a variety of tissues, whereas cytochrome *c* is a heme protein that is localized between the outer and inner mitochondrial membrane. Oxidative stress enhances mitochondrial dysfunction via the release of cytochrome *c*. Thus, the up-regulation or down-regulation of these genes might be related to mitochondrial dysfunction. Mitochondria dysfunction plays a prominent role in the induction of apoptosis.

6. Apoptosis

Apoptosis or programmed cell death plays an important role in tissue homeostasis and normal development [59]. Nonetheless, improper apoptosis has been implicated in neurodegenerative diseases such as AD [60,61]. Apoptosis of the hippocampal and cortical neurons negatively affects learning and memory [62,63].

Terminal deoxynucleotidyl transferase mediated dUTP nick end-labelling (TUNEL) is an assay for detecting DNA fragmentation, a main feature of apoptosis. A study showed that intraperitoneal injection of scopolamine at 2 mg/kg caused an increase in the number of TUNEL-positive cells by ~1.3-fold, indicating scopolamine induces neuronal apoptosis in the hippocampus of male Kunming mice [40]. At a lower dose of 0.3 mg/kg, scopolamine did not trigger neuronal apoptosis in the 7-day-old rat brain at 24 h [64]. Another study indicated that a higher dose of scopolamine (3 mg/kg, i.p.) increased apoptosis in hippocampal neurons of male Wistar rats [65]. Cellular shrinkage, a prominent morphological characteristic of apoptotic cell death, was also seen in scopolamine-treated SY-SH5Y cells [35]. Using DNA electrophoresis approach, scopolamine also induces DNA cleavage patterns, suggesting the involvement of apoptosis [43]. Flow cytometric assessment of apoptotic cell death using Annexin V-FITC dye also indicated that scopolamine-induced cytotoxicity in C6 cells is mediated via apoptosis [48].

Bax is a pro-apoptotic protein, whereas Bcl-2 is an important anti-apoptotic protein. Ajami et al. reported that scopolamine increased Bax and Bcl-2 protein expression in rats [42]. It is unclear why the anti-apoptotic protein Bcl-2 was elevated in that study. However, two other independent studies employing immunofluorescent staining and western blot analysis reported that higher Bax, but lower Bcl-2 protein expression levels in the brain of scopolamine-treated male Kunming mice [12,40]. Scopolamine also increases the ratio of Bax/Bcl-2 in hippocampus in these rodents [10,31].

Caspases play an essential role in inflammation and apoptosis. Caspases-3, -6 and -7 are the executioner caspases for apoptosis [66]. Caspases-8 and -9 are the initiator and usually occur as inactive procaspases [67]. Venkatesan et al. have demonstrated that scopolamine

induced caspase-3 activation by 1.6-fold in comparison to the control C6 glioma cells [48]. In vitro studies using Neuro2a neuroblastoma cells treated with 0.06 mg/ml scopolamine also showed an increase in caspase-3 activity by ~1.8-fold [30,34]. Scopolamine increased the cleaved caspase-3 expression in both cortex and hippocampus of male Kunming mice by ~1.8-fold [12]. In addition, scopolamine (1 mg/kg, i.p.) for 21 days also increased caspase-3 expression by ~1.4-fold and reduced procaspase-9 expression by half in homogenized brain tissues of female Wistar rats [13]. On top of that, caspase-3 activity was also increased in these animal models [30,40].

Caspase-3 is responsible for poly(ADP-ribose) polymerase (PARP) cleavage during apoptosis [68]. PARP uses nicotinamide adenine dinucleotide (NAD) as its substrate and catalyzes the poly(ADP-ribosyl)ation of many nuclear proteins. It has been proposed that PARP to contribute to cell death by depleting intracellular ATP and NAD [69,70]. It has been reported that scopolamine increased PARP protein expressions in homogenized brain tissues of female Wistar rats [13]. Seladin-1 inhibits the activity of caspase-3, a key regulator of apoptosis [71]. It has been demonstrated that seladin-1 gene is down-regulated in AD [72]. Treatment with scopolamine at 2 mg/kg/day for six weeks suppressed seladin-1 (~25%) mRNA levels to ~25% of the control value [19].

7. Neuroinflammation

Neuroinflammation is commonly found in the brain of AD patients [73]. Proinflammatory cytokines, including interleukin (IL)-1β, IL-4 and tumor necrosis factor (TNF)-α, released from microglia in the brain has been linked to AD progression [74]. A study by Demirci et al. demonstrated that scopolamine administration (1 mg/kg, i.p.) elevated IL-1β, IL-4 and TNF-α levels in the brain of female Wistar albino rats by > 15% in comparison to the control group [13], suggesting scopolamine does in fact mimic the AD picture. Intraperitoneal administration of scopolamine at the concentrations of 0.4 mg/kg/day (for a week) and 1 mg/kg/day (for 2 weeks) elevated the hippocampal TNF-α level by ~1.3-fold and ~2.5-fold, respectively, in male Wistar rats [20,75]. Scopolamine treatment (2 mg/kg/day, i.p.) for 6 weeks increased TNF-α (2.8-fold) and IL-1β (3.1-fold) and reduced IL-10 (0.4-fold) protein levels in the hippocampus of male Wistar rats as compared to the control.

Nitric oxide (NO) is a proinflammatory molecule that plays a pivotal role in AD. Excess levels of NO produced during inflammation can initiate neurodegeneration [76]. There are different isoforms of NO synthase. Inducible NO synthase (iNOS) is the isoform that contributes to the high level of NO production during inflammatory processes following induction by proinflammatory cytokines [77]. In addition, this molecule can also induce apoptosis and oxidative damage in neurons [78]. The level of NO was elevated in Aβ-treated BV2 microglial cells [79]. Treatment with scopolamine (1 mg/kg, i.p.) increased the production of NO by 1.25-fold in male Wistar rats [44]. Scopolamine administration at 3 mg/kg (i.p) elevated iNOS level by > 1.3-fold in the cortex and hippocampus of male Kunming mice [12].

p38 and c-Jun N-terminal kinase (JNK) are stress-activated kinases that belong to the mitogen-activated protein kinase (MAPK) family. It has been suggested that p38 and JNK mediate the induction of neuronal apoptosis in AD [80] and the progression of inflammatory-related diseases [81]. Scopolamine caused up-regulation of phosphorylated p38 and down-regulation of phosphorylated JNK in SH-SY5Y cells [35]. The mechanisms of scopolamine-induced down-regulation of phosphorylated JNK in SH-SY5Y cells are unclear and require further investigation. Nevertheless, it has been reported that JNK activation can promote survival of synoviocytes and B-lymphoma cells [82,83]. Thus, the ability of JNK in inducing both survival and apoptotic signaling pathways implies that the role of this kinase is complex.

8. Suppression of trophic factors

Growth or trophic factors, such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), have been shown to have neurogenic and neuroprotective effects. NGF, for example, contributes to the survival, maintenance and growth of mammalian neurons [84]. Recent studies on animal models have reported that NGF deprivation causes AD-like pathologies, such as Aβ accumulation, tau hyper-phosphorylation and synaptic dysfunction, and that NGF treatment can reverse those pathological changes [85]. Scopolamine reduced the hippocampal level of NGF by 38% [19]. In addition, scopolamine also attenuates the secretion of NGF in C6 cells and neurite outgrowth in N2a cells [48].

VEGF, a potent angiogenic factor, is involved in neuronal differentiation and proliferation, as well as neuroprotection and memory formation [86]. A study has reported VEGF is sequestered by Aβ protein in the AD brain leading to impaired neuroprotection [87]. Scopolamine reduced the levels of VEGF by 64% in the hippocampus area of male Wistar rats as compared to the control group [19].

BDNF has very important roles in neurogenesis, neuroprotection and synaptic plasticity [88,89]. BDNF mRNA and protein levels were low in postmortem AD brain samples [90]. The expression of BDNF gene can be regulated by extracellular signal-regulated protein kinase (ERK), a signaling molecule in the MAPK pathway, and cAMP response element binding protein (CREB), a transcription factor and a downstream signaling molecule of ERK [91]. CREB signaling is associated with memory augmentation, whereas disruption of its activity interrupts this process [92]. In differentiated SH-SY5Y cells, CREB mRNA and protein levels were reduced significantly by Aβ protein [93]. Meanwhile, scopolamine reduced phosphorylated CREB and BDNF to < 60% in the hippocampus and cortex of male ICR mice [36,47], indicating scopolamine mediates the cognitive deficit via suppressing CREB-BNDF neurotrophic pathway. Scopolamine also reduced the adult male Wistar rat hippocampal CREB mRNA expression by 33% [19]. Scopolamine down-regulated phosphorylated ERK, in hippocampus of male ICR mice [10] and SH-SY5Y cells [35].

Other than CREB, Akt signaling has also been associated with the neuroprotective and trophic effects of BDNF. BDNF increases Akt protein level while reducing phosphorylated Akt, the active form of Akt [94,95]. Akt signaling has also been implicated in AD pathology. For instance, phosphorylated Akt level was suppressed in Aβ-infused rat model of AD [96]. Moreover, Akt can also act as a regulator of tau pathology by promoting tau hyper-phosphorylation and phosphorylated tau accumulation [20]. Scopolamine administration (1 mg/kg/day) for 14 days elevated the total Akt level by > 2.5-fold, but reduced phosphorylated Akt to < 60% in comparison to the control, in the cortex and hippocampus of male Wistar rats [20]. In addition, scopolamine also reduced Akt phosphorylation in the mouse hippocampus [97].

9. Conclusions

Scopolamine induces a number of cellular alterations including impaired antioxidative defense system, increased oxidative stress, mitochondrial dysfunction, apoptosis and neuroinflammation. These pathological changes mimic those that are found in AD patients and other AD models. Thus, it can be concluded that scopolamine is a useful pharmacological tool to study the cellular and molecular changes that are related AD pathogenesis.

Declaration of Competing Interest

The author has no conflict of interest to report.

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