Chapter 11
Understanding Mechanism of Action of Herbal Drugs in Age Related Degenerative Brain Disorders

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Abstract In this review, we will briefly introduce preventive strategies represented by selected plants which are also popularly used by different traditional medicines and have scientific evidences of possible alternative therapeutic value for neuro-protection. Among these commonly used in most traditional medicines of China and India are *Gingko biloba*, *Panax ginseng*, *Curcuma longa*, *Withania somnifera* (WS) etc. In addition, scientific evidences of usefulness of *Vitis vinifera* (Grapes) as red wine, Coffee sp and *Camelia sinensis* (Tea) will be discussed. Here, we will also discuss in detail the possible use of WS as potential candidate for treatment of AD. Clinical trials and animal research supported the use of WS for treatment of anxiety, cognitive and neurological disorders, senile dementia, Alzheimer's (AD) and Parkinson's disease (PD), and as antioxidant and anti-inflammatory agent. This might be important in suggesting therapeutic implications of WS in neurodegenerative disorders.

Keywords AChE • *Withania somnifera* • Co-localization • Neurodegeneration • NADPH-d • Alzheimer's • Parkinson's disease

11.1 Introduction

Aging is a complex physiological process that involves both morphological and biochemical changes occurring, with the passage of time, in single cells and the whole organism. Among the many theories proposed to explain the mechanism of aging at the molecular level, the oxidative stress or free radical hypothesis has received wide support. Biochemically, oxidative stress is defined as a disturbance in the cell oxidation/reduction (redox) status, leading to the production of partially reduced oxygen intermediates, more reactive than molecular oxygen in its ground state, termed as

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reactive oxygen species (ROS). ROS production and oxidative damage to biomac-romolecules (nucleic acids, lipids and proteins) initiate the development of age-dependent degenerative diseases. This represents a condition in which the function and/or structure of affected tissues or organs progressively deteriorate over time, such as cardiovascular and neurodegenerative diseases. Neurodegenerative disease is mainly defined as a deterioration of the intellectual and cognitive faculties which are generally associated with aging and/or age associated disorders (AD, PD).

The human brain accounts for less than 2 % of the body weight, it consumes about 20 % of the oxygen available through respiration. Therefore, because of its high oxygen demand, the brain is the most susceptible organ to oxidative damage (Bajra 2004). Additionally, the high amount of polyunsaturated fatty acids (PUFAs) present in neuronal membranes makes the brain tissues particularly susceptible to lipid peroxidation reactions, resulting in the formation of cytotoxic aldehydes, such as malondialdhyde (MDA) and 4-hydroxynonenal (HNE) leading to cell degeneration.

11.2 Alzheimer's (AD) and Parkinson's (PD) Disease

Alzheimer's is today's the most prevalent disorder in the elderly population. It is characterized by progressive and irreversible memory loss, cognitive deterioration and personality changes, usually with an onset after 65 years of age. Though memory impairment appears in the early stages of the disease but motor and sensory functions are not affected until later stages. Parkinson's is the second most common age-related neurodegenerative disease. PD is a movement disorder characterized by resting tremors, bradykinesia, extrapyramidal rigidity and loss of postural reflexes i.e., disturbance in walking or equilibrium. Incidence rates of AD and PD increase exponentially with age. According to the World Health Organization (WHO), neurodegenerative diseases will become the world's second leading cause of death by the middle of this century, in fact overtaking cancer (Menken et al. 2000). It is also predicted that by 2050 the number of AD patients in the US only, could range from 11 to 16 million (Hebert et al. 2003). PD affects approximately 1 % of population aged 65–69 years and the prevalence increases to 3 % in the 80 year old or above group. According to an estimate, by 2030 in Western Europe, the number of PD cases will be double from 4.5 to 9 million and the prevalence varies in different ethnic and geographic groups (Dorsey et al. 2007).

11.3 Neuropathology of AD and PD

Although AD and PD differ in their clinical symptoms as well as disease course, but both are basically characterized by a progressive loss of neurons in different brain areas. Furthermore, they are characterized by the aggregation of intracellular pro-

teins (Shastry 2003) as the presence of extracellular senile plaques and intracellular accumulation of neurofibrillary tangles (NFTs) in the brain of AD patients has been observed. Senile plaques are composed of fibrillar amyloid β (Aβ) peptides produced by cleavage of the Aβ precursor protein (APP), whereas NFTs consist of hyperphosphorylated microtubule associated tau protein (Pβ). Selective neuronal loss is particularly severe in specific cerebral areas—the neocortex, hippocampus, limbic system and subcortical areas (Selkoe 2001). On the contrary, PD is characterized by the selective degeneration of dopaminergic neurons located in the pars compacta of the substantia nigra. One of the main neuropathological hallmarks of PD is the aggregation of the intracellular protein β -synuclein, to form intracytoplasmic inclusions (Lewy bodies) in these neurons (Agorogiannis et al. 2004).

The etiology of both the diseases is multifactorial, with a complex combination of genetic and non-genetic components. Oxidative damage is believed to be one of the leading cause of neuronal degeneration in both AD and PD (Nikam et al. 2009a, b). Though the exact mechanism of AD is still unknown, several lines of evidence suggest that oxidative stress is implicated in Aβ -induced neurotoxicity, besides apoptosis and inflammation. Studies have suggested a close relationship between cerebral biometal (Fe, Cu, Zn) and AD pathology. These redox-active metals and Aβ peptides interact to elevate oxidative stress in brain tissues. In PD, the mechanisms involved in selective degeneration of dopaminergic neurons in the nigrostriatal system are not clearly known, though evidence suggests that oxidative stress may arise from the metabolism of dopamine, producing free radical species (Jenner 2004). Compared to the rest of brain, the subtantia nigra pars compacta is exposed to a higher rate of ROS formation and to higher levels of oxidative stress (Yuan et al. 2007).

11.4   Current Pharmacological Intervention for AD and PD

There is no cure for AD and PD and current therapies provide only symptomatic improvement by either replacing the levels or controlling the metabolism of neurotransmitters involved in the disease or to restore their imbalance. Cholinesterase inhibitors are still the first line prescript drug available for patients with mild to moderate AD. By inhibiting the hydrolysis of acetylcholine in the synaptic cleft, these drugs restore the levels of the neurotransmitter in the affected neurons (Persson et al. 2009). Memantine was approved for moderate to severe cases, which acts as a specific, non- competitive N-methyl-D-aspartate (NMDA) receptor antagonist, counteract the excitotoxicity of glutamate, the major excitatory neurotransmitter in the brain (Terriot et al. 2004). Anti-inflammatory and antioxidant therapies were also proposed as possible preventive strategies. In case of PD, current therapeutic approach for the symptomatic treatment includes Levodopa (L-DOPA) which is converted to dopamine in the body, thus replenish the decreased dopamine levels in affected tissues (Lewitt 2008; Olanow et al. 2009).

11.5   Neuroprotective Strategies

The term neuroprotection refers to the strategies to protect the central nervous system (CNS) against neuronal injury due to trauma, stroke or aging resulting into chronic neurodegenerative changes in the nervous system. Such age related changes are characteristic of the AD and PD. Oxidative damage is believed to be one of the leading causes of such neuronal degeneration in both AD and PD. Among various current strategies used, herbal medicines represent valuable resource for antioxidant defence that can counteract the imbalance of the cell redox homeostasis and keep the ROS levels under the cytotoxic threshold. Antioxidant defenses also comprise vitamins and nonenzymatic scavengers abundant in food and medicinal plants (Prior and Cao 2000). As complementary or alternative therapy, herbal medicine refers to the medical use of plant organs—leaves, stems, roots, flowers, fruits and seeds for their curative properties. Herbal preparations contain complex mixture of several active components (phytochemicals), including phenylpropanoids, isoprenoids and alkaloids, which have different biological activities and responsible for its medicinal properties. It is often difficult to determine which component of the herbs has such neuroprotective biological activity (Suk 2005; Mclatchey et al. 2009).

11.5.1   Ginkgo biloba L.

Chinese term Ginkgo meaning silver apricot. The use of this plant in traditional medicine can be traced back to approximately 5,000 years to the origins of traditional community medicines (TCM). Modern Chinese pharmacopoeia has also introduced Ginkgo leaves as treatment for vascular insufficiency and to improve longevity. Since 1965, German physicians have prescribed *G. biloba* for the treatment of cognitive dysfunctions, dementia and AD. In the early 1970s, the standardized extract of *G. biloba* leaves EGb 761 was isolated and was widely prescribed in Europe and US for the symptomatic treatment of AD, cerebral insufficiency (a nonspecific age related deterioration of mental functions), improvement of cerebral blood flow and memory (Birks and Grimley 2009). EGb 761 contains 24 % of flavonoids and 6 % of terpenic lactones. The flavonoid fraction is composed of three flavonols—quercetin, keampferol and isorhamnetin, whereas terpenic derivatives are represented by diterpenic lactones, the ginkgolides A, B, C, J and M, and a sesquiterpenic trilactone, the bilobalide. Bilobalide can reduce damage caused by global brain ischemia and excitotoxicity-induced neuronal death (Chandrasekaran et al. 2003).

Neuroprotective action of EGb 761 is due to combination of antioxidative, anti-amyloidogenic and anti-apoptotic activities, by virtue of the blend of its bioactive phytochemicals (Yao et al. 2001; Luo et al. 2002). The neuroprotective efficacy of the extract was assessed by different clinical studies. In a randomized, double-blind, placebo-controlled trial of patients, treatment groups received over a 24-week

period an oral daily dose of 160 mg EGb 761 or 5 mg donepezil (a cholinesterase inhibitor), whereas the control group was treated with a placebo. Study (Syndrome Kurz Test, SKT, Mini-Mental State Examination, MMSE, and Clinical Global Impression, CGI) showed that both EGb 761 and donepezil were more effective in improving the cognitive function of patients with mild to moderate AD. In an analysis reviewing many randomized, double blind, placebo- controlled clinical studies, patients diagnosed with AD received 120–240 mg/day of EGb 761 for 3–6 months. Two phase III clinical trials, the GEM (Ginkgo Evaluation of Memory) study in US and the Guid Age study in France, focused on evaluation of EGb 761 efficacy in the prevention of AD in more than 3,000 patients older than 70 years. Both studies were randomized; double blind, placebo-controlled trials (DeKosky et al. 2006). In another study, EGb 761 was administered in a dose of 120 mg twice daily and the incidence of all-cause dementia was used as primary outcome. Secondary outcome included the rate of cognitive decline, the incidence of cardio- and cerebrovascular events and mortality. In another study, the efficacy of 240 mg daily dose of EGb 761 was evaluated, with the incidence of AD during a 5-year follow up period as primary outcome. This study was the largest clinical trial carried out in Europe on the prevention of AD (Vellas et al. 2006).

11.5.2   Panax ginseng

Panax ginseng (Ginseng) (Chinese rènshē n = man root) is another important herbal plant, refers to the shape of the root resembling the leg of a man. It is one of the most widely used herbs in TCM for boosting Qi (energy). It is an anti-aging herb, employed for thousands of years as a tonic and revitalizing agent. Several species within the Panax genus are growing in North- Eastern Asia. *P. ginseng* or Asian ginseng are among the most commonly used species in Korean traditional medicine and Japanese traditional medicine. Other important species are Vietnamese ginseng (*P. vietnamensis*), Siberian ginseng (*Eleutherococcus senticosus* Maxim. which is not a true ginseng) and American ginseng (*P. quinquefolius* L.) (Yun 2001). Ginseng root is characterized by the presence of ginsenosides (triterpenic saponin complexes). Ginseng is considered as an adaptogenic herb. Adaptogens are able to increase the body's resistance to stress, trauma, anxiety and fatigue by modulating the immune functions. Furthermore, it improves memory, learning performance and motor activity. Ginseng may provide protection against neurodegeneration by multiple mechanisms. In an experimental study, it was reported to attenuate Aβ and glutamate-induced toxicity, enhancing clearance of Aβ by stimulating the phagocytic activity of microglia and promoting neuron survival by increasing the levels of neurotrophic factors (Chen et al. 2006). These data show that ginseng acts on different stages of the neurodegenerative diseases. Studies in human subjects have also shown the efficacy of ginseng in treatment of AD. In a clinical trial, patients aged 50 years or older with mild to moderate AD related dementia were randomized into three groups, two treatment groups received an oral daily dose of 9 or 4.5 g

of Korean red ginseng for 12 weeks. The high-dose ginseng group showed scores higher than the control group ones, while differences between low-dose ginseng and placebo were not significant (Joo et al. 2008, Heo et al. 2008). Apart from AD, Ginseng has also shown protective effects against PD in several cell culture and animal studies. Both ginsenosides and root extracts are able to promote neuronal cell survival by reducing the neurotoxicity induced by toxins or parkinsonism mimetics, such as 1-methyl-4-phenyl-1,2,3,6-tetra- hydropyridine (MPTP) and its active metabolite 1-methyl-4-phenylpyridinium (MPP+) in rodents. These neurotoxins induce oxidative stress and lead to cell death of dopaminergic neurons, as in PD (Van Kempen et al. 2003). In other studies, ginsenosides, besides protecting neuronal cells, have shown neurotrophic effects promoting neurite overgrowth (Rudakewich et al. 2001).

11.5.3   *Curcuma longa L.*

Curcuma longa or Turmeric is the dried rhizome. It's a spice used in curry, and widely used as flavouring agent in many food preparations, particularly in India. The bright yellow colour of turmeric is due to curcumin, the main bioactive constituent. Turmeric has been used for thousands of years in Ayurvedic and Chinese medicine as well. Curcuminoids is a group of polyphenols including mainly curcumin, demethoxycurcumin and bisdemethoxycurcumin. Components of turmeric are currently undergoing scientific evaluation for numerous potential benefits due to their anti-inflammatory, antiproliferative, pro-apoptotic, antioxidant, antiviral and antidiabetic activity. Numerous molecular targets of curcumin have been identified over the years, including cyclooxygenase (COX)-2 and lipoxygenase (LOX) (Strimpakos and Sharma 2008).

Based on epidemiological studies, a hypothesis has been raised that the wide use of Curcuma sp. among Indians may be responsible for the significantly lower prevalence of AD in India compared to US. In a transgenic animal model of AD, supplementation with a low dose of curcumin (160 ppm) for 6 months reduced indices of both inflammation and oxidative stress. In particular, the levels of pro-inflammatory cytokine IL-1β, of oxidized proteins and of Aβ peptide decreased significantly (Linn et al. 2001). Anti- amyloidogenic activity of curcumin was extensively reported in in vitro and animal models (Uno et al. 2004). The possible binding of curcumin to the redox-active metals iron and copper suggests alternative neuroprotective mechanism of the substance (Yang et al. 2005). The combination of non-steroidal anti-inflammatory drugs (NSADs) and curcumin attenuated oxidative damage, cognitive deterioration and Aβ peptide deposition in both cell culture and animal model. In the same study, anti-inflammatory activity of curcumin was observed, due to the inhibition of cytokine production and microglia activation and increase of phagocytosis index (Cole et al. 2004). Because the process of inflammation plays a major (detrimental) role in the pathogenesis of the most chronic illnesses including neurodegenerative diseases, the therapeutic potential of curcumin

as anti-inflammatory agent in the prevention and treatment of chronic disorders has been recently highlighted (Aggarwal and Harikumar 2008). In fact, activation of microglial cells in CNS results in the production of pro-inflammatory mediators that propagate neuronal injury exacerbating neurodegenerative diseases. In rat, cur-cuminoid pigments suppressed NO production by LPS-activated microglia (Zhang et al. 2008). In a model of global cerebral ischemia, induced in Mongolian gerbils by transient occlusion of common carotid arteries, administration of curcumin by intraperitoneal injections (30 mg/kg body weight) for 2 months attenuated isch-emia-induced neuronal death and glial activation. The decrease of lipid peroxida-tion, mitochondrial dysfunction and apoptotic indices were other biochemical re-sponses mediated by curcumin.

11.5.4   *Vitis vinifera L. (Grape) and Red Wine*

Phytochemicals in Grape include phenylpropanoids, isoprenoids (responsible for the wine flavouring) and alkaloids (such as indolic compounds). Studies were re-cently focusing on the biological activity of selected grape polyphenols, such as the stilbene resveratrol and flavonoids (Pervaiz 2003). Population-based control studies have provided the substantial evidence that a regular (daily or possibly 3–4 times weekly) intake of moderate amounts (two glasses/day) of red wine (made from *V.vinifera*) is associated with a lower risk of developing dementia and AD (Pinder 2009). The Study evaluated the association between the type of alcoholic beverage and incidence of ischemic stroke, showing a protective effect of wine consumption among subject aged 60–69 years. In general, protective effects of grape polyphenols against neurodegenerative diseases can be ascribed to their anti-amyloidogenic, antioxidant and anti- inflammatory activity (Blanchet et al. 2008). The daily administration of resveratrol (50 or 100 mg/kg) for 1 or 2 weeks to adult male mice significantly prevented the nigrostriatal dopaminergic neuron deple-tion, after the acute treatment with the neurotoxin MPTP injected intraperitoneally (Rivière et al. 2008). In different cell lines stably transfected with human amyloid protein (APP), resveratrol was shown to promote the intracellular degradation of Aβ peptides via a mechanism that involves the proteasome, without direct inhi-bition of the enzymes β- and γ-secretases implicated in the Aβ protein synthesis. Neuroprotective effects of three major grape polyphenolic constituents (resveratrol, quercetin and catechin) were assessed in cultured mixed (glial/neuronal) cells of rat hippocampus. Treatment with polyphenols reduced the cytotoxicity induced by both the NO free radical donor sodium nitroprusside (SNP) and intracellular ROS accumulation (Bastianetto et al. 2000). In a mouse model of AD, the moderate con-sumption of Cabernet Sauvignon promoted the non-amyloidogenic processing of APP mediated by β-secretase, thereby preventing or delaying the generation of Aβ peptides. More recently, a grape seed polyphenolic extract significantly prevented Aβ protein oligomerization, by inhibiting the Aβ protein aggregation into high-mo-lecular-weight oligomeric Aβ species, both in vitro and in Tg 2,576 mice. Besides,

when orally administered to these animals, the extract attenuated the cognitive deterioration typical of AD.

11.5.5   Coffee (Coffea spp.)

Coffee is native to Yemen and Ethiopia. The genus Coffea (Rubiaceae family) includes two main species: *C. arabica* L., and *C. canephora* L. (syn. C. robusta L.). Caffeine, a methylxanthine, is the most important bioactive constituent of the coffee known to provide neuroprotection. Other structurally similar xanthine alkaloids are theophylline and theobromine, found primarily in tea and chocolate, respectively. As regards pharmacological activity, methylxanthines act as adenosine-receptor antagonists. In particular, caffeine is a nonspecific, competitive blocker of adenosine A1 and A2A receptors, distributed throughout the central nervous system (Cauli and Morelli 2005). According to human epidemiological studies, caffeine, as well as other adenosine A2A receptor antagonist, may play a role in preventing or delaying the onset of AD. A case control study involving subjects aged 50 years with probable diagnosis of AD and sex-matched controls found that individuals consuming two cups of coffee (approximately 200 mg of caffeine) per day for 20 years were at a significantly lower risk of developing the disease than those that consumed less caffeine (Maia and Mendonca 2002). These results were in accordance with previous studies in which coffee consumption was consistently protective against PD for men and women in the absence of estrogen therapy (Ascherio et al. 2001). It is noteworthy that acute intake of high doses of coffee (five cups of coffee, approximately 500 mg of caffeine, at one sitting) results in activation of stress responses, as demonstrated by increased plasma levels of cortisol, β -endorphin and epinephrine, in turn raising heart rate, blood pressure and releasing free fatty acids from storage.

11.5.6   Tea (Camellia sinensis Kuntze)

Three predominant types of tea: green, black and oolong are popular. Green tea is the least processed and thus provides the most antioxidant polyphenols, particularly catechins (epigallocatechin, epigallocatechin-3-gallate), flavonols (myricetin, quercetin, kaempherol) and proanthocyanidins (Khokhar and Magnusdottir 2002). Being catechins, particularly epigallocatechin- 3-gallate (EGCG), 10–20 times more concentrated than flavonols in normally brewed tea (Kuriyama et al. 2006), seem to be responsible for most of the health benefits of tea. Green tea drinkers appear to have lower risk for a wide range of diseases, from simple bacterial or viral infections to chronic degenerative conditions including cardiovascular disease, cancer and stroke (Coimbra et al. 2006). As regards protection against AD and PD, green tea catechins, until recently thought to work simply as antioxidants, are now known to invoke a wide spectrum of neuroprotective cellular mechanisms. These include iron chelation, scavenging of free radicals, activation of signaling pathways, and

regulation of mitochondrial function to avoid excessive production of free radicals (Mandel and Youdim 2004). As reported above, iron accumulation in specific brain areas and free radical damage to brain cells are considered the major damaging factors responsible for a wide range of neurodegenerative disorders including AD and PD. In the brain, epigallocatechin-3-gallate (EGCG) has been shown to act as an iron chelator, binding to and removing iron, thus preventing it from contributing to the production of free radicals. In addition to removing iron, EGCG also increases the activity of two major antioxidant enzymes, superoxide dismutase (SOD) and catalase, further helping to decrease free radical damage (Weinreb et al. 2004). Another active compound in green tea, epicatechin (EC), reduces the formation of β -amyloid protein and of the consequent plaque-like deposits in the brain, characteristic of AD. The protective effects of black and green tea extracts and their main constituents, epigallocatechin gallate and epicatechin gallate have been shown in an in vitro system of cultured neurons. In the presence of these extracts, neurons survived to the toxic effect of β -amyloid protein. Green tea polyphenols have also demonstrated the ability to affect cell signaling pathways, in particular the MAPK pathways, which are triggered by oxidative stress. MAPK signaling pathways in brain cells are thought to play a critical role in neurodegenerative diseases.

Although no human studies on AD have yet reported benefit from tea consumption, population studies have shown that simply consuming two or more cups of green tea daily reduces the risk of cognitive decline and PD. A study at Japan's Tohoku University, using a Mini-Mental State Examination (a well-accepted standardized test for measuring cognitive function) on 1,003 subjects over age 70, researchers showed that drinking more than two cups per day of green tea reduces chances of cognitive impairment in both men and women by 64 % (Haque et al. 2006). Those drinking green tea experienced significantly less mental decline than those drinking the least. In particular, those drinking more than two cups a day had 54 % lower risk of age- related decline in memory, orientation, and ability to follow commands and attention as compared with elderly Japanese who drank less than three cups a week. Those drinking four to six cups of green tea a week (one cup a day) had a 38 % lower risk of decline in brain functions. As reported in the previous section, tea also contains caffeine, although half that found in coffee (Khokhar and Magnusdottir 2002). The amount of caffeine that ends up in a cup of green tea varies according to the amount of tea used and the length of time the leaves are infused (Perva-uzunalic et al. 2006). When green tea is brewed, its caffeine combines with catechins in the water, reducing the caffeine's activity compared to coffee or cocoa. In addition, L-theanine, which is only found in tea plants and some mushrooms, directly stimulates the production of alpha brain waves, calming the body while promoting a state of relaxed awareness.

11.5.7   *Withania somnifera Dunal (WS)*

Withania somnifera Dunal (WS) is known as Ashwagandha or Indian ginseng. It has been commonly used in Indian traditional medicines for over 3,000 years and

categorized as Rasayana drug in Ayurveda. Rasayna drugs are known to augment defense against diseases, arrest aging, revitalize the body in debilitated condition, increase the capability of the individual to resist adverse environmental factors and create a sense of mental wellbeing. Animal research (Bhatnagar et al. 2005; Gupta and Rana 2007; Bhatnagar 2009) supported the use of WS for treatment of anxiety, cognitive and neurological disorders, senile dementia, Alzheimer's and Parkinson's disease and as antioxidant and anti-inflammatory agent. It has also been used to treat stress, insomnia and age related disorders including neurodegeneration (Gupta et al. 2003; Mishra et al. 2000). The biologically active constituents in WS are alkaloids (ashwagandhin, cuscohygrine, anahygrine, topine etc.), steroidal compounds, including ergostane type steroidallactones, withaferin A, withanolides A–Y, withasomniferin A, withasomnidienone, withasomnierose A–C, withanone etc. Other constituents include saponins containing an additional acyl group (Sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X) (Ganzera et al. 2003). It has been reported that phenolic compounds present in the root of WS contribute to the overall antioxidant activity of the plant (Prakash et al. 2007). Our laboratory (Jain et al. 2001, Bhatnagar et al. 2009), has reported neuroprotective actions of WS root extract which were correlated with its antioxidant property and ability to inhibit lipid peroxidation both in vitro and in vivo. Bhattacharya et al. (2000) suggested that glycowithanolides present in WS protect against lipid peroxidation due to its antioxidant action. In addition, withanolides and sitoindosides (VII–X) also enhance catalase and glutathione peroxidase activities in rat frontal cortex and striatum (Bhattacharya et al. 2001).

11.6   Neuroprotective Mechanism of *Withania somnifera*

In this review, we have mainly discussed possible neuroprotective mechanism of WS in the brain degenerative disorders. In the brain, peroxynitrite is a strong non radical prooxidant produced during metabolism of nitric oxide (NO), which mediates neuronal damage. NO, synthesized by the enzyme, neuronal nitric oxide synthase (nNOS) is a neuromodulator in hippocampus and cortex. nNOS forms NO from L-arginine in the presence of molecular oxygen. Several earlier reports have indicated that nNOS is identical to neuronal NADPH diaphorase (NADPH-d). Histochemical localization of NADPH-d staining in mouse hippocampus fully coincides with both histochemical NADPH-d and immunocytochemical nNOS staining in rat hippocampus. In the hippocampus, a number of NADPH-d positive cell bodies and dense fibers are present in CA1, CA2 and CA3 subfields and also in the oriens, pyramidal layer and stratum radiatum. In the dentate gyrus as well, number of NADPH-d positive neurons are found in polymorphic layer (Matsushita et al. 2001). Small amount of NO synthesized during neuronal activity mediates diverse physiological functions which include neuronal differentiation, neuronal survival, and synaptic plasticity. Excessive production of NO has also been implicated in a number of pathophysiological conditions including stress (Mcleod et al. 2001) and

glutamate mediated neurotoxicity. De Oliveira et al. (2000) reported that exposure to stress can lead to enhanced nNOS expression, which is a glutamate mediated process. Neurotoxic effects of NO depend upon its redox state and are likely to be mediated by its free radical nature. NO reacts readily with superoxide ($O2-$) to produce peroxynitrite ($ONOO-$), which can oxidize iron–sulphur clusters, zinc fingers and protein thiols, thereby contributes to cellular energy depletion (Stamler 1994). In hippocampus, activity of nNOS is mainly regulated by glutamate and serotonin (Harwey et al. 2006). Release of glutamate and serotonin is modulated by glucocorticoids (GC); thus GC can activate nNOS by altering release of these two neurotransmitters. GC play defensive role during stress, but sustained elevated level of GC during chronic stress can lead to neurodegeneration. Hippocampus is most vulnerable to GC induced neurodegeneration because of presence of high concentration of GC receptors. Several studies suggest that inhibitors of the neuronal NO synthesizing enzyme (nNOS) may be useful as neuroprotective agents in treatment of neurodegenerative diseases. Considering the neuroactive properties of WS root extract and to understand the possible mechanism of neuroprotection, we studied the effects of WS root extract on NADPH-d activity, Choline acetyl transferase (ChAT) activity, serotonin level in hippocampus and serum GC level in mice exposed to chronic restraint stress. Results showed that exposure to restraint stress can lead to activation of significant proportion of NADPH-d positive neurons, decrease in ChAT activity and serotonin level in the hippocampus. Stress also elevated cortisone level. These effects of stress were significantly reversed in animals treated with WS root extract.

Hippocampus is known to mediate stress, learning and memory, LTP and antidepressant behavioral effects (Joca and Guimaraes 2006). Stress induced production of NO in hippocampus can negatively alter above functions. NO also facilitates the release of several neurotransmitters including gamma-aminobutyric acid (GABA), glutamate, biogenic amines and neuropeptides (Prast and Philipu 2001). Thus NO can modulate neuronal excitability, firing and neurotransmitter release. Excessive production of NO in brain and other tissues mediates injury in diverse disease states. NO toxicity is likely to be mediated by its free radical nature. NO can readily react with superoxide ($O2-$) to produce peroxynitrite ($ONOO-$) which mediates much of the toxic effects of NO. It is well reported that various physiological and physical stressors produce changes in the expression of nNOS in different brain areas. Bhatnagar et al. (2009) showed, a significant increase in nNOS positive neurons in hippocampus is shown in adult mice exposed to chronic restraint stress. The rational behind using restraint stress was the reported evidences that restraint stress can potentially stimulate nNOS in other brain regions as well viz., limbic region, hypothalamus and dorsal raphe nucleus (Echeverry et al. 2004; Masood et al. 2004; Okere and Waterhouse 2006). Generation of NO by nNOS is regulated directly or indirectly, by at least three other neurotransmitters i.e., glutamate, serotonin and acetylcholine. Glutamate by acting on NMDA receptor causes Ca^{++} influx and activates nNOS as NO generation is a Ca^{++}/Calmodulin dependent process. NMDA receptor and nNOS are found to be linked physically through a molecular scaffold protein PSD-95. Stress induces glutamate release in the brain and excitotoxic neurodegen-

erative effect of glutamate in chronic stress is mediated by increased expression of nNOS. It has been suggested that, in hippocampus, nNOS is under tonic inhibition by serotonergic neurons. An increased nNOS activity was observed when 5-HT2 receptor was inhibited by ritanserin in rats exposed to forced swimming (Harwey et al. 2006). Furthermore, serotonin depletion may result in excessive production of NO and neurodegeneration. Release of both, glutamate and serotonin is altered by glucocorticoids (corticosterone in rodents). Excessive production of glutamate during stress is associated with glucocorticoid release and stress induced elevation in extracellular glutamate release is attenuated by adrenalectomy. Hippocampal neurons are particularly sensitive to glucocorticoid level and are involved in termination of corticosterone secretion at the end of stress due to the presence of both type I and type II glucocorticoid receptors. Activity of glutamatergic neurons is also regulated by cholinergic neurons. In a study, exogenous ACh added to superfusion fluid inhibited the Ca^{++} dependent K^+ evoked release of glutamate in a concentration dependent manner in rat hippocampus. It has been proposed that impaired serotonin level during stress is mediated by hypercortisolemia, acting on the high concentration of glucocorticoid receptors in the hippocampus (Campbell and Macqueen 2004). It has also been suggested that stress induced changes in hippocampal glutamate mechanism may precede changes in serotonin function (McEwen et al. 2002).Taking together these findings, we postulate that nNOS inhibitory property of WS is possibly mediated by suppression of glucocorticoid release and activation of cholinergic neurons and not by acting on glutamatergic or NOergic neurons. It has been reported that defined extract of WS do not directly affect the glutamatergic markers. Treatment with defined extract from WS does not affect NMDA and AMPA glutamate receptor subtypes in any cortical or sub cortical regions. Sitoindosides VII–X and withaferin A have been shown to modulate brain functions by binding with cholinergic receptors in the rat. WS extract can also reverse the reduction in cholinergic markers in rats (Bhattacharya and Muruganandam 2003). Schliebs et al. (1997) also suggested that defined extract from WS may affect preferentially, events in the cortical and basal forebrain cholinergic signal transduction cascade. Treatment with WS extract upregulates cortical muscarinic acetylcholine receptor expression. Both, low glucocorticoid level and acetylcholine release, in turn downregulates activity of glutamatergic neurons in the hippocampus while, stress induced impairment of serotonin release is attenuated by low glucocorticoid level. Modulation of release of these three neurotransmitters i.e., acetylcholine, glutamate and serotonin by WS in all probability contributes to inhibition of nNOS in extract treated stressed mice. Our results support this postulate as we have observed decreased glucocorticoid level and increased ChAT activity following WS treatment in stressed animals. These results confirm and extend previous findings and explain the nNOS inhibitory properties of WS. In summary, it is proposed that neuroprotective properties of WS are owing to neurochemical alterations of specific neurotransmitter systems. WS extract can also suppress glucocorticoid release in chronic stress. This purported new role of WS can be exploited for treatment of neurodegenerative disease like Alzheimer's, which is caused by a decline in ACh level as well as in oxidative stress. WS can inhibit the production of free radicals like peroxynitrite.

Bhatnagar et al. (2009) have investigated the effects of fresh leaf juice of WS on enzymes acetylcholinesterase (AChE) and nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) activity in vivo and in vitro to support our earlier observations, and also to understand the potential therapeutic mechanism of WS in AD or related diseases. We reported that WS root extract can increase ACh level by increasing ChAT expression, and serotonin level in hippocampus (Bhatnagar et al. 2009). ACh in turn inhibits NO producing enzyme NADPH-d. The grey side of this study was that effect of WS extract on AChE was not studied. Also there was a lack of data on correlation between distribution of cholinergic neurons and NOergic neurons in various fore brain regions including hippocampus to support our hypothesis that ACh inhibits NADPH-d activity. Study revealed that WS fresh leaf juice significantly inhibits not only AChE activity but also NADPH-d activity which is clearly evident from histochemical, biochemical and in vitro observations. Counting of NADPH-d positive cell bodies in these areas also showed significant reduction in number of NADPH-d positive cell bodies in WS treated brain when compared with control brain. To prove that WS inhibits both AChE and NADPH-d, we attempted to co-localize both AChE and NADPH-d in same tissue sections. Results showed that AChE and NADPH-d cell bodies co-localizing both the enzymes not only showed reduced reactive enzyme intensity but also their number in forebrain areas after WS treatment as compared to cell bodies positive for either AChE or NADPH-d.

Reduced AChE activity thus suggested increased ACh, while reduced NADPH-d activity demonstrates decrease in NO. To further confirm that elevated ACh inhibits NO production, in vitro study was carried out. Primary hippocampal cell culture was exposed to glutamate in the presence and absence of ACh and then nitrite production was measured (Fig. 11.1).

Glutamate was used to elevate production of NO in cultured cells. It is well documented that nNOS is physically linked to NMDA glutamate receptors and activation of nNOS is positively correlated with NMDA receptor activation. Results showed significantly low nitrite level in glutamate treated cells which were incubated with ACh as compared to cell which were not. This confirms that NO production decreases in the presence of ACh.

One of the characteristics of AD is cholinergic deficit (Talesa 2001; Melo et al. 2003). Post-mortem studies have shown that AD brains are characterized by low ChAT, while AChE, a principal cholinesterase in brain which hydrolyzes endogenous neurotransmitter Ach (Talesa 2001), was shown to be increased within and around amyloid plaques to promote the assembly of amyloid beta peptides (Aβ) in the fibrils and to increase the toxicity of the peptides. Thus most accepted strategies for treatment of AD is the use of cholinesterase inhibitors. Inhibition of AChE results in increase in ACh level, which led to functional improvement of cholinergic synapses, reduced neuronal degeneration and enhanced regional synthesis of neurotrophic molecules. Thus many plant extracts—Ginkgo and Ashwagandha not names of the extracts but only plants which characteristically inhibit cholinesterase have been used as drug against early symptoms of AD in traditional medicinal system (Vinutha et al. 2007; Chowdhry et al. 2004).

Fig. 11.1 Flow chart to
explain possible mechanism
of neuroprotective action of
Withania somnifera (WS)
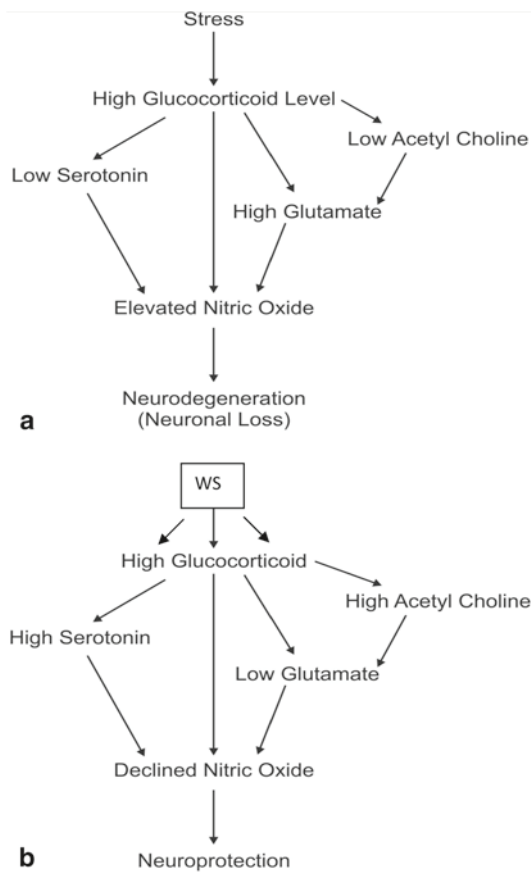
NO is also an intra and extra-cellular mediator of cell functions and is diffusible free radical cellular messenger. Kluchova et al. (2000) suggested that NO may play a role in control of cholinergic neuronal activity at synapses. Law et al. (2001a) have shown that aberrantly expressed nNOS in brain results in rise in NO that can be toxic due to its free radical properties. Law et al. (2001b) have also shown that Aβ increases NO release which decreases neuronal viability. In our study, as neurons showing co-localization of both the enzymes are less in WS treated brain as compared to control brain, a possible regulatory interaction of WS on AChE and NADPH-d can be envisaged. Based on these studies, we have suggested a mechanism of the inhibitory effects of WS.

11.7   Conclusions

Post-mortem studies in AD patient brain have shown clear links between the disease and deficiency of neurotransmitter Acetylcholine (ACh). In AD along with chronic shortage of ACh, high Nitric oxide (NO) was also reported. High NO is deleterious

to neuronal cells and contributes to disease progression. Until recently, reversal of the deficiency by alleviating the level of neurotransmitters, use of agonists or the inhibition of the enzymes involved in the removal of the neurotransmitter at synapses, are the treatments used for AD and related diseases. But these treatments are not ideal and provide only symptomatic relief. A variety of natural products or their derivatives have shown the desired effects and some of these have been brought into clinical use to treat various degenerative disorders. Though the presence of receptors or transporters for phytochemicals in brain tissues remains to be ascertained, compounds with multiple targets appear as a potential and promising class of therapeutics for the treatment of CNS diseases. Our results are in agreement that WS inhibits AChE and thereby enhances cholinergic neurotransmission in cortical and basal forebrain areas. Study also suggests that WS directly inhibits AChE but inhibition of NADPH-d is indirect. WS mediated inhibition of both AChE and NADPH-d could have therapeutic implications in AD which is characterized by reduced ACh and elevated NO.

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