

Current Topics

Pharmacologically Active Constituents from Plants Used in Traditional Medicine

Effects of Ashwagandha (Roots of *Withania somnifera*) on Neurodegenerative DiseasesTomoharu Kuboyama,^a Chihiro Tohda,^a and Katsuko Komatsu^{*b}^aDivision of Neuromedical Science, Institute of Natural Medicine, University of Toyama; and
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Neurodegenerative diseases commonly induce irreversible destruction of central nervous system (CNS) neuronal networks, resulting in permanent functional impairments. Effective medications against neurodegenerative diseases are currently lacking. Ashwagandha (roots of *Withania somnifera* DUNAL) is used in traditional Indian medicine (Ayurveda) for general debility, consumption, nervous exhaustion, insomnia, and loss of memory. In this review, we summarize various effects and mechanisms of Ashwagandha extracts and related compounds on *in vitro* and *in vivo* models of neurodegenerative diseases such as Alzheimer's disease and spinal cord injury.

Key words Ashwagandha; *Withania somnifera*; withanolide; denosomin; Alzheimer's disease; spinal cord injury

1. INTRODUCTION

In neurodegenerative diseases such as Alzheimer's disease, spinal cord injury, Parkinson's disease, and Huntington's disease, destruction of neuronal networks is a critical cause of the functional impairment. In neurodegenerative diseases, neuronal networks are believed not spontaneously regenerated by distinct causes; for example, amyloid β ($A\beta$) induces axonal atrophy in Alzheimer's disease, and inhibitory proteoglycans inhibit regeneration of axons in spinal cord injury.^{1–10} Although several drugs are clinically used for the treatment of neurodegenerative diseases, most of these drugs are for symptomatic treatment; drugs for fundamental cure of neurodegenerative diseases are not clinically available and greatly needed.

Ashwagandha (roots of *Withania somnifera* DUNAL) is one of the most valuable herbal drugs used in Indian traditional medicine (Ayurveda) as a *rasayana* drug that is capable of imparting long life, youthful vigor, and good intellectual powers.¹¹ Ashwagandha is clinically used for the treatment of general debility, consumption, nervous exhaustion, insomnia, loss of memory, and so on.^{12,13} These traditional uses imply that Ashwagandha may possibly be useful at improving neurodegenerative diseases. Indeed, this herbal drug has been reported to exert various pharmacological effects such as anti-inflammatory, anti-tumor, anti-oxidant, immunomodulatory, and anti-neuropsychiatric disease effects.^{14,15} In this review, we describe effects of Ashwagandha extracts, constituents of Ashwagandha (mainly withanolides: steroidal lactones with ergostane skeleton), and its derivatives in the context of neurodegenerative diseases.

2. ALZHEIMER'S DISEASE

2.1. Axon Regeneration and Synaptic Reconstruction In Alzheimer's disease, the formation of $A\beta$ deposits in the brain is widely accepted to be a critical cause of axonal atrophy and synaptic degeneration.^{1,2,16} We propose that promotion of axonal and synaptic regeneration may lead to reconstruction of neuronal networks and fundamental recovery from Alzheimer's disease. Therefore the effects of methanol extracts of Ashwagandha on neurite outgrowth using an *in vitro* culture system were investigated; methanol extract of Ashwagandha showed neurite outgrowth-promoting activity in human neuroblastoma SK-N-SH cells.¹⁷ Withanolide A, withanoside IV, and withanoside VI (Fig. 1) were identified as active constituents from the methanol extract that induced neurite outgrowth in human neuroblastoma SH-SY5Y cells and rat cortical neurons.^{18,19} An *in vitro* axonal atrophy model was established using an active partial fragment of $A\beta$ such as $A\beta_{25-35}$.²⁰ $A\beta_{25-35}$ induced axonal atrophy as potently as full-length $A\beta_{1-42}$.²¹ Withanolide A, withanoside IV, and withanoside VI were individually treated to the neurons displaying axonal atrophy. Each of these 3 compounds induced axonal growth even in the presence of $A\beta_{25-35}$.^{21–23} Subsequently, an *in vitro* synaptic degeneration model was established. Rat cortical neurons formed synapses *in vitro* during 21-d culture. Thereafter, $A\beta_{25-35}$ was treated to the neurons, resulting in losses of densities of presynapses and postsynapses.²¹ Nonetheless, post treatment with withanolide A, withanoside IV, or withanoside VI increased the synaptic densities. These 3 compounds were then tested *in vivo*. $A\beta_{25-35}$ was intracerebroventricularly (i.c.v.) injected to mice brains. Densities of axons and synapses in the parietal cortex were reduced, and spatial memory of the mice was diminished by i.c.v. injection of $A\beta_{25-35}$. Consecutive oral administration of withanolide A, withanoside IV, or withanoside VI for 12 d

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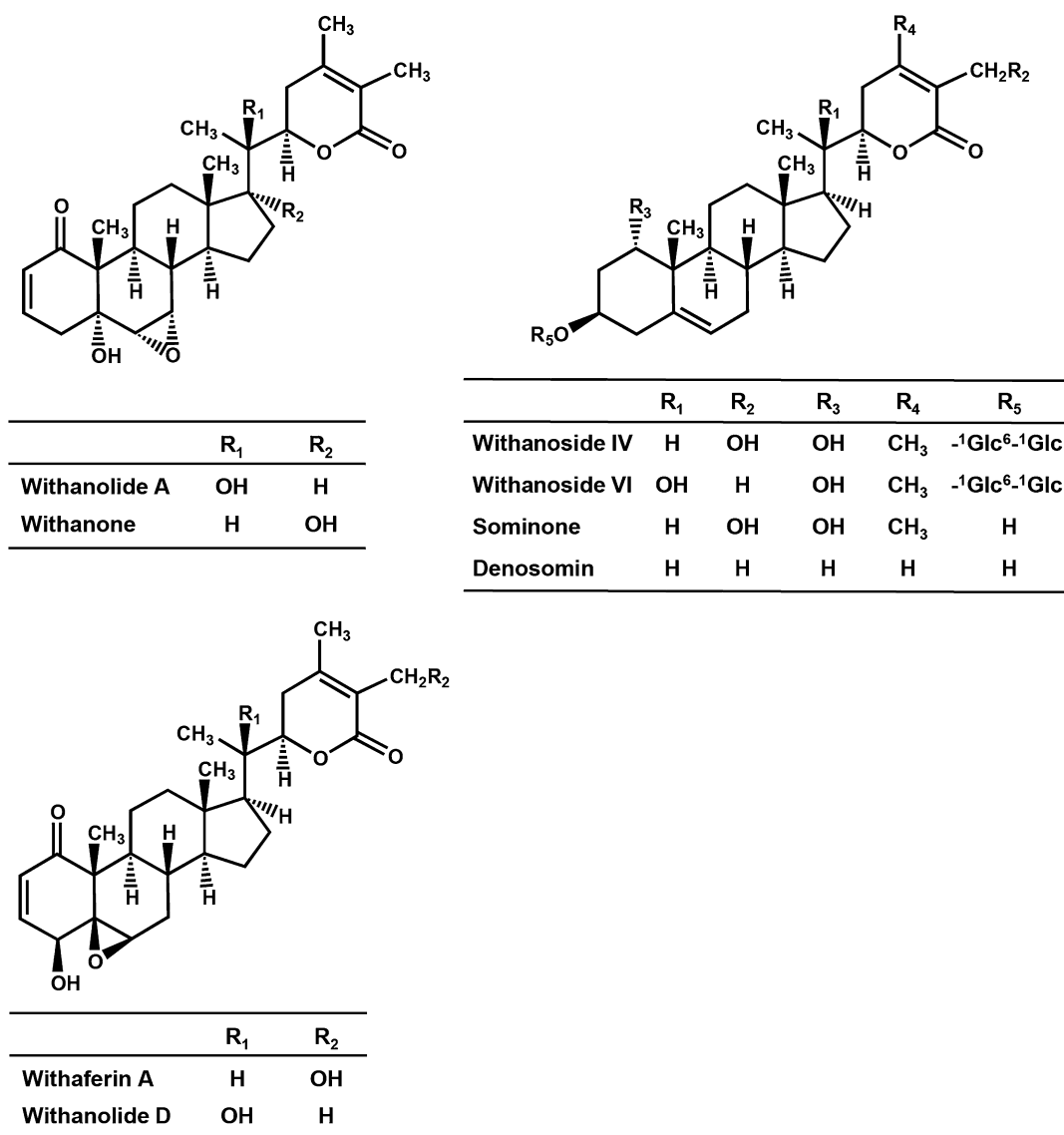


Fig. 1. Structures of Constituents of Ashwagandha and Derivatives from Withanoside IV

increased the densities of axons and synapses in the parietal cortex and improved spatial memory deficit.

Withanoside IV conjugates 2 glucoses at position C3. It was reported that, after oral administration, several glycosides of natural products were deglycosylated by human intestinal bacteria and subsequently absorbed in the blood.^{24,25)} Therefore orally administered withanoside IV was speculated deglycosylated at position C3 by the intestinal bacteria. After oral administration of withanoside IV to mice, serum was collected and analyzed by liquid chromatography/mass spectrometry.²²⁾ As a result, withanoside IV itself was not detected in the serum whereas sominsonone, an aglycon of withanoside IV, was detected (Fig. 1). Sominsonone induced axonal regeneration and synaptic reconstruction in A β 25–35-treated cortical neurons *in vitro*. In Alzheimer's disease model 5XFAD mice,²⁶⁾ a single intraperitoneal (i.p.) administration of sominsonone increased axonal density in the brain and improved object recognition memory impairment.²⁷⁾ These results suggest that withanoside IV is metabolized to its active principle sominsonone after oral administration, which subsequently induces marked recovery of neurites, synapses, and memory. These data suggest that

withanoside IV and its metabolite sominsonone are potential drugs against Alzheimer's disease.

In normal adult mice, a single i.p. administration of sominsonone increased axonal density in the brain and enhanced object location memory.²⁸⁾ Rearranged during transcription (RET) phosphorylation was increased in the brain by sominsonone-treatment. RET is a part of the receptor complex for glial cell-line-derived neurotrophic factor (GDNF) and is phosphorylated by GDNF stimulation.^{29,30)} Experimental knockdown of RET inhibited sominsonone-induced axonal growth in cultured cortical neurons.²⁸⁾ GDNF secretion was not influenced by treatment with sominsonone in cultured cortical neurons. These results indicate that sominsonone activates RET and induces axonal growth, possibly leading to memory enhancement.

Other groups have reported effects of withanolide A on Alzheimer's disease experimental models. Patil *et al.*³¹⁾ reported that withanolide A decreased beta-site amyloid precursor protein cleaving enzyme 1 (BACE1; known as β -secretase) expression and increased a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10; known as α -secretase) expression in cultured normal rat cortical neurons. A β is

produced from amyloid precursor protein (APP) by processing with BACE1 and presenilin 1 (PS1; known as γ -secretase).³²⁾ When APP is processed by ADAM10, $A\beta$ production is attenuated whereas non-toxic soluble APP α is alternatively produced. As a result, withanolide A increased soluble APP α production in the cultured neurons. It was also shown that withanolide A increased expression levels of insulin-degrading enzyme (IDE), a major proteolytic enzyme involved in $A\beta$ degradation. These results suggest that withanolide A possibly reduces $A\beta$ by increasing soluble APP α production and $A\beta$ clearance. Considering these reports, withanolide A is an important candidate as a multifunctional drug against Alzheimer's disease.

2.2. Neuroprotective Effects Kurapati *et al.*³³⁾ investigated effects of methanol–chloroform (3:1) extract of Ashwagandha against $A\beta$ 1–42-induced toxicity in cultured human neuroblastoma SK-N-MC cells. $A\beta$ 1–42 induced cell death and internalization of $A\beta$ 1–42. Simultaneous treatment with the extract and $A\beta$ 1–42 inhibited the above phenomena induced by $A\beta$ 1–42. Expression level of peroxisome proliferator-activated receptor- γ (PPAR γ) was decreased by $A\beta$ 1–42 and reversed by simultaneous treatment with the extract. The authors indicated that upregulation of PPAR γ by the extract supports neuroprotective effects against $A\beta$, but a causal relation between PPAR γ expression and neuroprotective effects was not clarified.

Effects of the alcoholic extract of Ashwagandha leaves (i-Extract)³⁴⁾ on scopolamine-induced cell damages were investigated by Konar *et al.*³⁵⁾ Scopolamine is a muscarinic receptor antagonist that induces amnesia in rodents.³⁶⁾ Scopolamine also influences expression of genes related to muscarinic receptor signaling pathways, apoptosis, and cell differentiation in the rat brain.³⁷⁾ Konar *et al.* showed that scopolamine-treatment induced cell deaths in cultured human neuroblastoma IMR32 cells and cultured rat glioma C6 cells, whereas i-Extract-treatment before scopolamine-treatment protected cells from death.³⁵⁾ Scopolamine treatment induced DNA damage and oxidative stress in C6 cells, whereas i-Extract treatment prevented those. Withaferin A and withanone (Fig. 1) were focused as major constituents in i-Extract. Although withanone showed preventive effects comparable to i-Extract, withaferin A did not. The authors concluded that withanone is a predominant active constituent in i-Extract that protects cells against scopolamine-induced damage probably *via* its anti-oxidative effects.

2.3. Clearance of $A\beta$ Sehgal *et al.*³⁸⁾ reported the effects of an authenticated Ashwagandha product serially extracted with chloroform-methanol (Arya Vaidya Sala, Kottakkal, India). In APP/PS1 Alzheimer's disease model transgenic mice,³⁹⁾ consecutive oral administration of the Ashwagandha extract for 30d reversed behavioral memory deficits in the radial arm task and decreased $A\beta$ level in the cerebral cortex and hippocampus but increased that in the blood plasma. Low-density lipoprotein receptor-related protein (LRP) in the liver and soluble form of LRP (sLRP) in plasma were upregulated by administration of the Ashwagandha extract. LRP mediates efflux of $A\beta$ from the brain into the periphery.^{40,41)} LRP in the liver is cleaved and released into the blood, where it remains present as sLRP.⁴²⁾ Liver-specific knockdown of LRP blocked both sLRP and $A\beta$ increment in plasma and $A\beta$ reduction in the brain after administration of the extract.³⁸⁾ Therefore the

Ashwagandha extract promotes $A\beta$ clearance in the brain *via* upregulation of liver LRP.

3. SPINAL CORD INJURY

In the injured region of the spinal cord, astrocytes are increased and form a glial scar where inhibitory proteoglycans are secreted from the astrocytes. Such deposit of inhibitory proteoglycans interferes with axonal regeneration.^{5,8–10)} Methylprednisolone sodium succinate is the only available medication for acute spinal cord injury; however, this drug is now less widely used because of very moderate efficacy coupled with recognized side effects. We attempted to induce axonal growth in injured spinal cord, which could lead to functional recovery. In our study, withanoside IV showed activity to induce axonal growth in the degenerated central nervous system.²²⁾ Therefore the effects of withanoside IV on spinal cord-injured mice were investigated.⁴³⁾ One hour after contusion injury at the L1 spinal cord, consecutive oral administration of withanoside IV was started. Administration of withanoside IV for 21d induced axonal growth in the spinal cord and recovered hindlimb motor function.

After oral administration, withanoside IV is metabolized into sominone in the intestine as described above, and sominone is thought an active principle for axonal growth activity.²²⁾ We synthesized a novel compound “denosomin” that is an analogous derivative of sominone⁴⁴⁾ (Fig. 1). In cultured cortical neurons, denosomin showed axonal growth activity comparably to sominone. Synthesis of denosomin is easier and more efficient than that of sominone. Therefore we investigated the effects of denosomin on spinal cord-injured mice.⁴⁵⁾ One hour after contusion injury at the L1 spinal cord, consecutive oral administration of denosomin was started. Administration of denosomin for 14d recovered hindlimb motor function. At that time, axonal growth was facilitated by administration in the injured center; additionally glial fibrillary acidic protein-positive reactive astrocytes also increased in the injured center. In astrocyte pure culture, denosomin increased astrocytic proliferation and inhibited H₂O₂-induced astrocytic death. Conditioned medium isolated from denosomin-treated astrocytes induced axonal growth in cultured cortical cells; nonetheless the conditioned medium did not contain denosomin. We identified that vimentin, an intermediate filament protein, was increased in cultured astrocytes after denosomin-treatment, and denosomin increased secretion of vimentin from cultured astrocytes. Although functions of the secreted vimentin on neurons had not been identified, an effect of extracellular vimentin on axonal growth was confirmed in cultured cortical cells. Also *in vivo*, denosomin-administration increased vimentin-positive astrocytes but not inhibitory proteoglycan-positive ones in the injured region of the spinal cord-injured mice. These results indicate that denosomin increases vimentin-secreting astrocytes in the injured region, leading to axonal growth and recovery of motor function in spinal cord-injured mice. In spinal cord injury, astrocytes in the glial scar were thought a major impediment to axonal growth.^{5,8–10)} However, upregulation and secretion of vimentin converts this burden into a force for functional recovery from spinal cord injury.

Table 1. Effects of Ashwagandha Extracts, Constituents, and Derivatives on Neurodegenerative Diseases

Disease models	Materials	Functions
Alzheimer's disease/Dementia	Extract	Neurite outgrowth <i>in vitro</i> ¹⁷⁾ Memory improvement ³⁸⁾ Neuroprotective effects <i>in vitro</i> ^{33,35)} $A\beta$ clearance <i>in vivo</i> ³⁸⁾
	Withanolide A	Neurite outgrowth <i>in vitro</i> ^{18,19)} Axonal regeneration and synaptic reconstruction <i>in vitro</i> and <i>in vivo</i> ²¹⁾ Memory improvement ²¹⁾ Upregulation of BACE1, ADAM10 and IDE <i>in vitro</i> ³¹⁾
	Withanoside IV	Neurite outgrowth <i>in vitro</i> ^{18,19)} Axonal regeneration and synaptic reconstruction <i>in vitro</i> and <i>in vivo</i> ²²⁾ Memory improvement ²²⁾
	Withanoside VI	Neurite outgrowth <i>in vitro</i> ^{18,19)} Axonal regeneration and synaptic reconstruction <i>in vitro</i> and <i>in vivo</i> ²³⁾ Memory improvement ²³⁾
	Sominone	Axonal regeneration and synaptic reconstruction <i>in vitro</i> ²²⁾ Axonal growth <i>in vivo</i> ²⁸⁾ Memory improvement ²⁷⁾ and memory enhancement ²⁸⁾
	Denosomin Withanone	Axonal regeneration and neuroprotective effect <i>in vitro</i> ⁴⁵⁾ Neuroprotective effects <i>in vitro</i> ³⁵⁾
Spinal cord injury	Withanoside IV Denosomin	Axonal growth <i>in vivo</i> and functional recovery ⁴³⁾ Axonal growth <i>in vivo</i> and functional recovery ⁴⁵⁾
Parkinson's disease	Extract	Anti-oxidant effect <i>in vivo</i> ⁵⁵⁻⁵⁹⁾ Neuroprotective effect <i>in vivo</i> ^{55,59)} Functional recovery ⁵⁵⁻⁵⁹⁾
Huntington's disease	Extract	Anti-oxidant effect <i>in vivo</i> ⁶⁰⁾ Functional recovery ⁶⁰⁾

4. SAFETY OF ASHWAGANDHA AND ITS CONSTITUENTS

Considering activities against neurodegenerative diseases, clinical application of Ashwagandha and its constituents is expected to improve such diseases. Some groups have previously reported safety of Ashwagandha and its constituents. Administration of water extract of Ashwagandha (100 mg/kg/d) with drinking water for 8 months did not show any toxicity in rats.⁴⁶⁾ Consecutive oral administration of 80% methanol extract of Ashwagandha (2000 mg/kg/d for 28 d) also showed no toxicity.⁴⁷⁾ However, oral administration of the alcoholic extract from defatted seeds of Ashwagandha induced acute toxicity in mice: LD₅₀ was 1750±41 mg/kg.⁴⁸⁾ Following i.p. administration of an ethanol extract of Ashwagandha, LD₅₀ was 1259 mg/kg in mice.⁴⁹⁾

Although withanolide A, withanoside IV, and withanoside VI showed neurite outgrowth activity at 1 μ M, withaferin A and withanolide D (Fig. 1) showed neurotoxicity at 1 μ M in cultured SH-SY5Y cells^{18,19)} and normal cortical neurons.²³⁾ Withaferin A and withanolide D are known to elicit anti-tumor effects.⁵⁰⁻⁵²⁾ Other reports also showed that withaferin A revealed cytotoxic activities in normal and cancer cells⁵³⁾ and did not show anti-Alzheimer's disease effects.^{18,19,23,35)} When i.p. administered, LD₅₀ of withaferin A in mice was approximately 80 mg/kg.⁵⁴⁾

Considering that Ashwagandha has been used in Ayurveda since ancient times, traditional use of Ashwagandha including some detoxification methods might show no severe toxicity. However, considering the toxicity of several of its constituents, toxic compounds contents in Ashwagandha should be

taken into account particularly when used in high doses.

5. CONCLUSION

Ashwagandha and its constituents showed various activities against models of Alzheimer's disease and spinal cord injury. Ashwagandha extracts also showed ameliorative effects against other neurodegenerative disease models such as Parkinson's disease and Huntington's disease,⁵⁵⁻⁶⁰⁾ suggesting that Ashwagandha may be useful against various neurodegenerative diseases (Table 1). Additionally, a novel drug candidate for spinal cord injury was synthesized as a derivative of constituents of Ashwagandha. These results suggest that Ashwagandha is a potential basis for novel drugs against neurodegenerative diseases. In addition, pharmacological analyses of Ashwagandha extracts and related compounds gave us novel insights for the treatment of neurodegenerative diseases; for instance, modulation of astrocyte properties can lead to recovery from functional impairment in spinal cord injury, and peripheral organ-targeted effects can lead to clearance of $A\beta$ in the brain. However, direct target molecules of Ashwagandha-related compounds have not been identified as yet. Clarification of these issues may lead to identification of novel targets for therapy of neurodegenerative diseases. Further studies of Ashwagandha will probably contribute to resolving an urgent unmet medical need: efficacious treatments that may offer cure of neurodegenerative diseases.

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REFERENCES

- Dickson TC, Vickers JC. The morphological phenotype of beta-amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience*, **105**, 99–107 (2001).
- Perl DP. Neuropathology of Alzheimer's disease. *Mt. Sinai J. Med.*, **77**, 32–42 (2010).
- Kanaan NM, Pigino GF, Brady ST, Lazarov O, Binder LI, Morfini GA. Axonal degeneration in Alzheimer's disease: when signaling abnormalities meet the axonal transport system. *Exp. Neurol.*, **246**, 44–53 (2013).
- McKeon RJ, Schreiber RC, Rudge JS, Silver J. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. *J. Neurosci.*, **11**, 3398–3411 (1991).
- Silver J, Miller JH. Regeneration beyond the glial scar. *Nat. Rev. Neurosci.*, **5**, 146–156 (2004).
- Jones LL, Tuszynski MH. Spinal cord injury elicits expression of keratan sulfate proteoglycans by macrophages, reactive microglia, and oligodendrocyte progenitors. *J. Neurosci.*, **22**, 4611–4624 (2002).
- Imagama S, Sakamoto K, Tauchi R, Shinjo R, Ohgomori T, Ito Z, Zhang H, Nishida Y, Asami N, Takeshita S, Sugiura N, Watanabe H, Yamashita T, Ishiguro N, Matsuyama Y, Kadomatsu K. Keratan sulfate restricts neural plasticity after spinal cord injury. *J. Neurosci.*, **31**, 17091–17102 (2011).
- Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G, Silver J. Regeneration of adult axons in white matter tracts of the central nervous system. *Nature*, **390**, 680–683 (1997).
- Tom VJ, Steinmetz MP, Miller JH, Doller CM, Silver J. Studies on the development and behavior of the dystrophic growth cone, the hallmark of regeneration failure, in an *in vitro* model of the glial scar and after spinal cord injury. *J. Neurosci.*, **24**, 6531–6539 (2004).
- Yiu G, He Z. Glial inhibition of CNS axon regeneration. *Nat. Rev. Neurosci.*, **7**, 617–627 (2006).
- Sivarajan VV, Balachandran I. *Ayurvedic drugs and their plant sources*. International Science Publisher, New York (1994).
- Warrier PK, Nambiar VPK, Ramankutty C. *Indian Medicinal Plants: A Compendium of 500 Species*. Orient Longman, Madras, India (1996).
- Usmanghani K, Saeed A, Alam MT. *Indusynic medicine: traditional medicine of herbal, animal, and mineral origin in Pakistan*. Dept. of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan (1997).
- Kulkarni SK, Dhir A. *Withania somnifera*: an Indian ginseng. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **32**, 1093–1105 (2008).
- Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern. Med. Rev.*, **5**, 334–346 (2000).
- Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.*, **30**, 572–580 (1991).
- Tohda C, Kuboyama T, Komatsu K. Dendrite extension by methanol extract of Ashwagandha (roots of *Withania somnifera*) in SK-N-SH cells. *Neuroreport*, **11**, 1981–1985 (2000).
- Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C, Komatsu K. Withanolide derivatives from the roots of *Withania somnifera* and their neurite outgrowth activities. *Chem. Pharm. Bull.*, **50**, 760–765 (2002).
- Kuboyama T, Tohda C, Zhao J, Nakamura N, Hattori M, Komatsu K. Axon- or dendrite-predominant outgrowth induced by constituents from Ashwagandha. *Neuroreport*, **13**, 1715–1720 (2002).
- Yankner BA, Dawes LR, Fisher S, Villa-Komaroff L, Oster-Granite ML, Neve RL. Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science*, **245**, 417–420 (1989).
- Kuboyama T, Tohda C, Komatsu K. Neuritic regeneration and synaptic reconstruction induced by withanolide A. *Br. J. Pharmacol.*, **144**, 961–971 (2005).
- Kuboyama T, Tohda C, Komatsu K. Withanoside IV and its active metabolite, sominone, attenuate A beta(25–35)-induced neurodegeneration. *Eur. J. Neurosci.*, **23**, 1417–1426 (2006).
- Tohda C, Komatsu K, Kuboyama T. Scientific basis for the anti-dementia drugs of constituents from Ashwagandha (*Withania somnifera*). *J. Trad. Med.*, **22**, 176–182 (2005).
- Akao T, Hayashi T, Kobashi K, Kanaoka M, Kato H, Kobayashi M, Takeda S, Oyama T. Intestinal bacterial hydrolysis is indispensable to absorption of 18 beta-glycyrrhetic acid after oral administration of glycyrrhizin in rats. *J. Pharm. Pharmacol.*, **46**, 135–137 (1994).
- Tawab MA, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M. Degradation of ginsenosides in humans after oral administration. *Drug Metab. Dispos.*, **31**, 1065–1071 (2003).
- Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J. Neurosci.*, **26**, 10129–10140 (2006).
- Joyashiki E, Matsuya Y, Tohda C. Sominone improves memory impairments and increases axonal density in Alzheimer's disease model mice, 5XFAD. *Int. J. Neurosci.*, **121**, 181–190 (2011).
- Tohda C, Joyashiki E. Sominone enhances neurite outgrowth and spatial memory mediated by the neurotrophic factor receptor, RET. *Br. J. Pharmacol.*, **157**, 1427–1440 (2009).
- Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiovaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, Sariola H, Pachnis V. GDNF signalling through the Ret receptor tyrosine kinase. *Nature*, **381**, 789–793 (1996).
- Trupp M, Arenas E, Fainzilber M, Nilsson AS, Sieber BA, Grigoriou M, Kilkenny C, Salazar-Gruoso E, Pachnis V, Arumäe U, Sariola H, Saarma M, Ibáñez CF. Functional receptor for GDNF encoded by the *c-ret* proto-oncogene. *Nature*, **381**, 785–789 (1996).
- Patil SP, Maki S, Khedkar SA, Rigby AC, Chan C. Withanolide A and asiatic acid modulate multiple targets associated with amyloid-beta precursor protein processing and amyloid-beta protein clearance. *J. Nat. Prod.*, **73**, 1196–1202 (2010).
- Prox J, Rittger A, Saftig P. Physiological functions of the amyloid precursor protein secretases ADAM10, BACE1, and presenilin. *Exp. Brain Res.*, **217**, 331–341 (2012).
- Kurapati KR, Atluri VS, Samikkannu T, Nair MP. Ashwagandha (*Withania somnifera*) reverses beta-amyloid1–42 induced toxicity in human neuronal cells: Implications in HIV-associated neurocognitive disorders (HAND). *PLoS ONE*, **8**, e77624 (2013).
- Widodo N, Kaur K, Shrestha BG, Takagi Y, Ishii T, Wadhwa R, Kaul SC. Selective killing of cancer cells by leaf extract of Ashwagandha: identification of a tumor-inhibitory factor and the first

- molecular insights to its effect. *Clin. Cancer Res.*, **13**, 2298–2306 (2007).
- 35) Konar A, Shah N, Singh R, Saxena N, Kaul SC, Wadhwa R, Thakur MK. Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells. *PLoS ONE*, **6**, e27265 (2011).
 - 36) Klinkenberg I, Blokland A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neurosci. Biobehav. Rev.*, **34**, 1307–1350 (2010).
 - 37) Hsieh MT, Hsieh CL, Lin LW, Wu CR, Huang GS. Differential gene expression of scopolamine-treated rat hippocampus-application of cDNA microarray technology. *Life Sci.*, **73**, 1007–1016 (2003).
 - 38) Sehgal N, Gupta A, Valli RK, Joshi SD, Mills JT, Hamel E, Khanna P, Jain SC, Thakur SS, Ravindranath V. *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proc. Natl. Acad. Sci. U.S.A.*, **109**, 3510–3515 (2012).
 - 39) Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fulop L, Penke B, Zilberter Y, Harkany T, Pitkanen A, Tanila H. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. *J. Neurosci.*, **29**, 3453–3462 (2009).
 - 40) Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of Alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood–brain barrier. *J. Clin. Invest.*, **106**, 1489–1499 (2000).
 - 41) Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi N, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE, Zlokovic BV. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron*, **43**, 333–344 (2004).
 - 42) von Arnim CA, Kinoshita A, Peltan ID, Tangredi MM, Herl L, Lee BM, Spoelgen R, Hshieh TT, Ranganathan S, Battey FD, Liu CX, Bacskai BJ, Sever S, Irizarry MC, Strickland DK, Hyman BT. The low density lipoprotein receptor-related protein (LRP) is a novel beta-secretase (BACE1) substrate. *J. Biol. Chem.*, **280**, 17777–17785 (2005).
 - 43) Nakayama N, Tohda C. Withanoside IV improves hindlimb function by facilitating axonal growth and increase in peripheral nervous system myelin level after spinal cord injury. *Neurosci. Res.*, **58**, 176–182 (2007).
 - 44) Matsuya Y, Yamakawa Y, Tohda C, Teshigawara K, Yamada M, Nemoto H. Synthesis of sominone and its derivatives based on an RCM strategy: discovery of a novel anti-Alzheimer's disease medicine candidate “denosomin.” *Org. Lett.*, **11**, 3970–3973 (2009).
 - 45) Teshigawara K, Kuboyama T, Shigyo M, Nagata A, Sugimoto K, Matsuya Y, Tohda C. A novel compound, denosomin, ameliorates spinal cord injury via axonal growth associated with astrocyte-secreted vimentin. *Br. J. Pharmacol.*, **168**, 903–919 (2013).
 - 46) Sharma S, Dahanukar S, Karandikar SM. Effects of long-term administration of the roots of Ashwagandha (*Withania somnifera*) and Shatavari (*Asparagus racemosus*) in rats. *Indian Drugs*, **23**, 133–139 (1986).
 - 47) Prabu PC, Panchapakesan S, Raj CD. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. *Phytother. Res.*, **27**, 1169–1178 (2013).
 - 48) Singh N, Nath R, Lata A, Singh SP, Kohli RP, Bhargava KP. *Withania somnifera* (Ashwagandha), a rejuvenating herbal drug which enhances survival during stress (an adaptogen). *Int. J. Crude Drug Res.*, **20**, 29–35 (1982).
 - 49) Sharada AC, Solomon FE, Devi PU. Toxicity of *Withania somnifera* root extract in rats and mice. *Int. J. Pharmacog.*, **31**, 205–212 (1993).
 - 50) Vanden Berghe W, Sabbe L, Kaileh M, Haegeman G, Heyninc K. Molecular insight in the multifunctional activities of Withaferin A. *Biochem. Pharmacol.*, **84**, 1282–1291 (2012).
 - 51) Vyas AR, Singh SV. Molecular targets and mechanisms of cancer prevention and treatment by withaferin A, a naturally occurring steroidal lactone. *AAPS J.*, **16**, 1–10 (2014).
 - 52) Mondal S, Mandal C, Sangwan R, Chandra S, Mandal C. Withanolide D induces apoptosis in leukemia by targeting the activation of neutral sphingomyelinase-ceramide cascade mediated by synergistic activation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. *Mol. Cancer*, **9**, 239 (2010).
 - 53) Vaishnavi K, Saxena N, Shah N, Singh R, Manjunath K, Uthayakumar M, Kanaujia SP, Kaul SC, Sekar K, Wadhwa R. Differential activities of the two closely related withanolides, Withaferin A and Withanone: bioinformatics and experimental evidences. *PLoS ONE*, **7**, e44419 (2012).
 - 54) Sharada AC, Solomon FE, Devi PU, Udupa N, Srinivasan KK. Antitumor and radiosensitizing effects of withaferin A on mouse Ehrlich ascites carcinoma *in vivo*. *Acta Oncol.*, **35**, 95–100 (1996).
 - 55) Ahmad M, Saleem S, Ahmad AS, Ansari MA, Yousuf S, Hoda MN, Islam F. Neuroprotective effects of *Withania somnifera* on 6-hydroxydopamine induced Parkinsonism in rats. *Hum. Exp. Toxicol.*, **24**, 137–147 (2005).
 - 56) RajaSankar S, Manivasagam T, Sankar V, Prakash S, Muthusamy R, Krishnamurti A, Surendran S. *Withania somnifera* root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. *J. Ethnopharmacol.*, **125**, 369–373 (2009).
 - 57) Sankar SR, Manivasagam T, Krishnamurti A, Ramanathan M. The neuroprotective effect of *Withania somnifera* root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. *Cell. Mol. Biol. Lett.*, **12**, 473–481 (2007).
 - 58) RajaSankar S, Manivasagam T, Surendran S. Ashwagandha leaf extract: a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. *Neurosci. Lett.*, **454**, 11–15 (2009).
 - 59) Prakash J, Yadav SK, Chouhan S, Singh SP. Neuroprotective role of *Withania somnifera* root extract in maneb-paraquat induced mouse model of parkinsonism. *Neurochem. Res.*, **38**, 972–980 (2013).
 - 60) Kumar P, Kumar A. Possible neuroprotective effect of *Withania somnifera* root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. *J. Med. Food*, **12**, 591–600 (2009).