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Ashwagandha in brain disorders: A review of recent developments

Sultan Zahiruddin[1], Parakh Basist[1], Abida Parveen, Rabea Parveen, Washim Khan, Gaurav, Sayeed Ahmad*

Bioactive Natural Product Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, 110062, India

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ABSTRACT

Ethnopharmacological relevance: *Withania somnifera* (Family: Solanaceae), commonly known as Ashwagandha or Indian ginseng is distributed widely in India, Nepal, China and Yemen. The roots of plant consist of active phytoconstituents mainly withanolides, alkaloids and sitoindosides and are conventionally used for the treatment of multiple brain disorders.

Aim of the review: This review aims to critically assess and summarize the current state and implication of Ashwagandha in brain disorders. We have mainly focussed on the reported neuroactive phytoconstituents, available marketed products, pharmacological studies, mechanism of action and recent patents published related to neuroprotective effects of Ashwagandha in brain disorders.

Materials and methods: All the information and data was collected on Ashwagandha using keywords "Ashwagandha" along with "Phytoconstituents", "Ayurvedic, Unani and Homeopathy marketed formulation", "Brain disorders", "Mechanism" and "Patents". Following sources were searched for data collection: electronic scientific databases such as Science Direct, Google Scholar, Elsevier, PubMed, Wiley On-line Library, Taylor and Francis, Springer; books such as AYUSH Pharmacopoeia; authentic textbooks and formularies.

Results: Identified neuroprotective phytoconstituents of Ashwagandha are sitoindosides VII–X, withaferin A, withanosides IV, withanols, withanolide A, withanolide B, anaferine, beta-sitosterol, withanolide D with key pharmacological effects in brain disorders mainly anxiety, Alzheimer's, Parkinson's, Schizophrenia, Huntington's disease, dyslexia, depression, autism, addiction, amyotrophic lateral sclerosis, attention deficit hyperactivity disorder and bipolar disorders. The literature survey does not highlight any toxic effects of Ashwagandha. Further, multiple available marketed products and patents recognized its beneficial role in various brain disorders; however, very few data is available on mechanistic pathway and clinical studies of Ashwagandha for various brain disorders is scarce and not promising.

Conclusion: The review concludes the results of recent studies on Ashwagandha suggesting its extensive potential as neuroprotective in various brain disorders as supported by preclinical studies, clinical trials and published patents. However vague understanding of the mechanistic pathways involved in imparting the neuroprotective effect of Ashwagandha warrants further study to promote it as a promising drug candidate.

1. Introduction

Mental health problem and neurological disorders are a serious public health concern globally with more than one billion sufferers worldwide. For neurological disorders, modern medicine offers symptomatic treatment that is expensive and associated with several side effects. Natural products have been broadly exploited as an important source for medicine. A large number of medicines are derived from plant-based extractions and fractionation and, have great importance for humans. Nowadays, medical practitioners are more inclined towards natural medicines for a trustworthy treatment with cost effectiveness and lower incidence of side effect.

Ayurveda a popular Indian system of medicine, has a well-developed course of action for the management and treatment of brain associated disorders. A list of about 450 Ayurvedic medicinal plants, 56 popular plant or one of their ingredients of Ayurvedic prescriptions are available for neurological disorders (Balkrishna and Misra, 2018). One of the traditional well-known Indian medicinal plant is Ashwagandha (*Withania somnifera*) which is a common ingredient of several Ayurvedic formulations marketed for the treatment of neurological

* Corresponding author.
E-mail address: sahmad_jh@jamiahamdard.ac.in (S. Ahmad).
[1] Equal contribution.

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Table 1
Marketed herbal formulation of Ashwagandha in brain disorders.

S.N.	Name of the formulation	Uses	Manufacture
1	Stresswin	Relieve stress, Antidepressant, Memory enhancement	Baidyanath Ayurved Bhawan
2	Stresscom	Relieve stress, Antidepressant	Dabur India Ltd.
3	Ashwagandharista	Memory enhancement, Sleep induction	Baidyanath Ayurved Bhawan
4	Himalaya Ashwagandha	Antistress	The Himalaya Drug Company
5	Amrukha kasturi	Neuroasthenia, Convalescence	Pankajakasthuri Herbal India Pvt. Ltd
6	Nature Ashwagandha	Antistress	Nature Ayurveda
7	Caruso's Ashwagandha	Antistress, Antianxiety	Caruso's Natural Health
8	Smrutihills	Memory enhancement, Antistress	Herbal Hills
9	Brento	Nervine tonic	Zandu Pharmaceutical Works Ltd
10	Stress Shield	Antistress, Antianxiety	Cureveda
11	Golden Milk	Memory enhancement	Gaia Herb

disorders. The herbal marketed formulations are summarized in Table 1.

Ashwagandha belongs to the family Solanaceae. Other common names of Ashwagandha are Indian ginseng, poison gooseberry and winter cherry. Ashwagandha is cultivated in North western and central part of India. In India Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan and Uttar Pradesh are the main producing state of Ashwagandha. It is also found in Nepal, China and Yemen. The climatic conditions required for the cultivation of Ashwagandha include an altitude of 1500 m above the sea level. The semi tropical regions which receive about 500–800 mm annual rainfall are the best suited for its cultivation. The crops require dry condition during growing periods and optimum temperature required for its cultivation is 20–38 °C. The sandy loam or light red soil and partial shade sun are other suitable factors for its growth (Kulkarni and Dhir, 2008; Mirjalili et al., 2009; Dar et al., 2015).

The extract of roots contains steroidal lactones with ergostane, which contain withanone, withaferin, withanolides, sitoindosides and about 0.2% alkaloids. Various studies have been conducted on active phytoconstituents which helps in providing a rationale background for drug design with upgraded and better pharmacological properties. The herb is reported to possess beneficial effects in a wide range of neurological disorders including stress, Parkinson's disease, Huntington's disease and Alzheimer's disease etc. Ashwagandha modulates the brain oxidative stress makers, such as superoxide dismutase (SOD), catalase, lipid peroxidation (LPO), and non-enzymatic antioxidants like glutathione (GSH). The roots and its extract induce axon and dendrite outgrowth, proposing its possible effect on neuronal regeneration (Durg et al., 2015).

Ashwagandha on the basis of phyto-pharmacological studies proved potential as anti-inflammatory, anti-oxidant, anti-cancer, anti-microbial, anti-malarial, diuretic, sedative, immunomodulatory and cardioprotective properties (John, 2014).This study aims to explore and evaluate the literature pertaining to the role of Ashwagandha in neurological disorders. Further, the present piece of work compiles neurological information of Ashwagandha extracts and its isolated compounds used in the neurological research studies and latest published patents. INSERT Table 1.

2. Phytoconstituents of Ashwagandha

Ashwagandha contains various non-nutritional chemicals, responsible for medicinal properties. More than 35 phytochemicals are isolated and identified in Ashwagandha. Main chemical constituents are alkaloids and steroidal lactone. Somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxy-tropane, cuscohygrine and anaferine are the alkaloids present in Ashwagandha, with withanine being the major one. The main steroidal lactones are withaferin A, withanolides A-Y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone (Gupta and Rana, 2007). The major phytoconstituents of Ashwagandha are depicted in Fig. 1 and their relative properties in brain disorders are summarized in Table 2.

3. Historical background of Ashwagandha

Traditional medicinal system is the potential therapeutic option to treat wide range of disorders with limited side effects, improved efficacy and thus owing property to manage the disease at different levels including prevention and cure. Ashwagandha is an important herb in various traditional system of medicine prolifically Ayurveda, Unani, Siddha, Homeopathy, Chinese, Tibetan, African, etc. In *Withania somnifera*, “somnifera” is a Latin word which means “sleep-inducer”, thus justifying its use extensively as neuroprotective. It is known by different local names in different parts of India.

The traditional Indian medicinal system (Ayurveda) and Chinese medicinal system are the most ancient systems. Ayurveda required more evidences and scientific-based research (Patwardhan et al., 2005). In Ayurveda, it is known as Ashwagandha, which means “odour of the horse” as it roots resemble sweaty horse. It has been extensively used from past thousand years back to treat wide range of diseases. Ayurvedic practitioners traditionally use it by boiling the fresh roots in milk. It also administered by crushing the roots into fine powder called as “churna” and blending it with fluids mainly water, milk, honey. Other parts including leaves, shoots, seeds and berries have also been used to improve health and increase longevity. In Ayurvedic system, it is classified as “Rasayana” means “tonic” and mainly act as body rejuvenator, defense against disease, slows aging and enhances memory (Samadi, 2013; Forman and Kerna, 2018).

Unani is a traditional form of medicine system mainly in Middle East and South Asian countries. It is based on the principle of equilibrium, and therapies employ physical means for disease curing. It employs administration of either specific diet or modulation in quality and quantity of food. Natural substances either single drugs or combination of two or more drugs are used for treating ailments (Shiddamallayya et al., 2016). Withania somnifera is known as Asgand in Unani system and is described in book “*Kitab-ul-Hashaish*”. According to literature *Asgand Nagori* and *Asgand Dakani* are the two varieties known in Unani system, however *Asgand Nagori* is medically more preferred (Uddin et al., 2012). It is used either as a single drug or in combination. Various Unani formulations are available containing *Withania somnifera* such as habbe Asgand, kushta gaodanti, majoon salab and majoon zanjabeel (Anonymous, 2007). It is used for the treatment of polyarthritis, rheumatoid arthritis, lumbago, painful swellings, spermatorrhoea, asthma, leucoderma, general debility, sexual debility, anxiety neurosis, scabies, ulcers and leucorrhoea (Anonymous, 2007; Khare, 2007; Nadkarni, 1982).

Tibetan system of medicine (TSM) is one of the world's oldest known traditional systems of medicine. It is popular mainly in northern part of India i.e. Ladakh, Lahul and Spiti and is followed by Amchis, herbal doctors (Shah, 1982). It is based upon the literature of Indian Buddhist system and is also known as Sowa-Rigpa which refers to

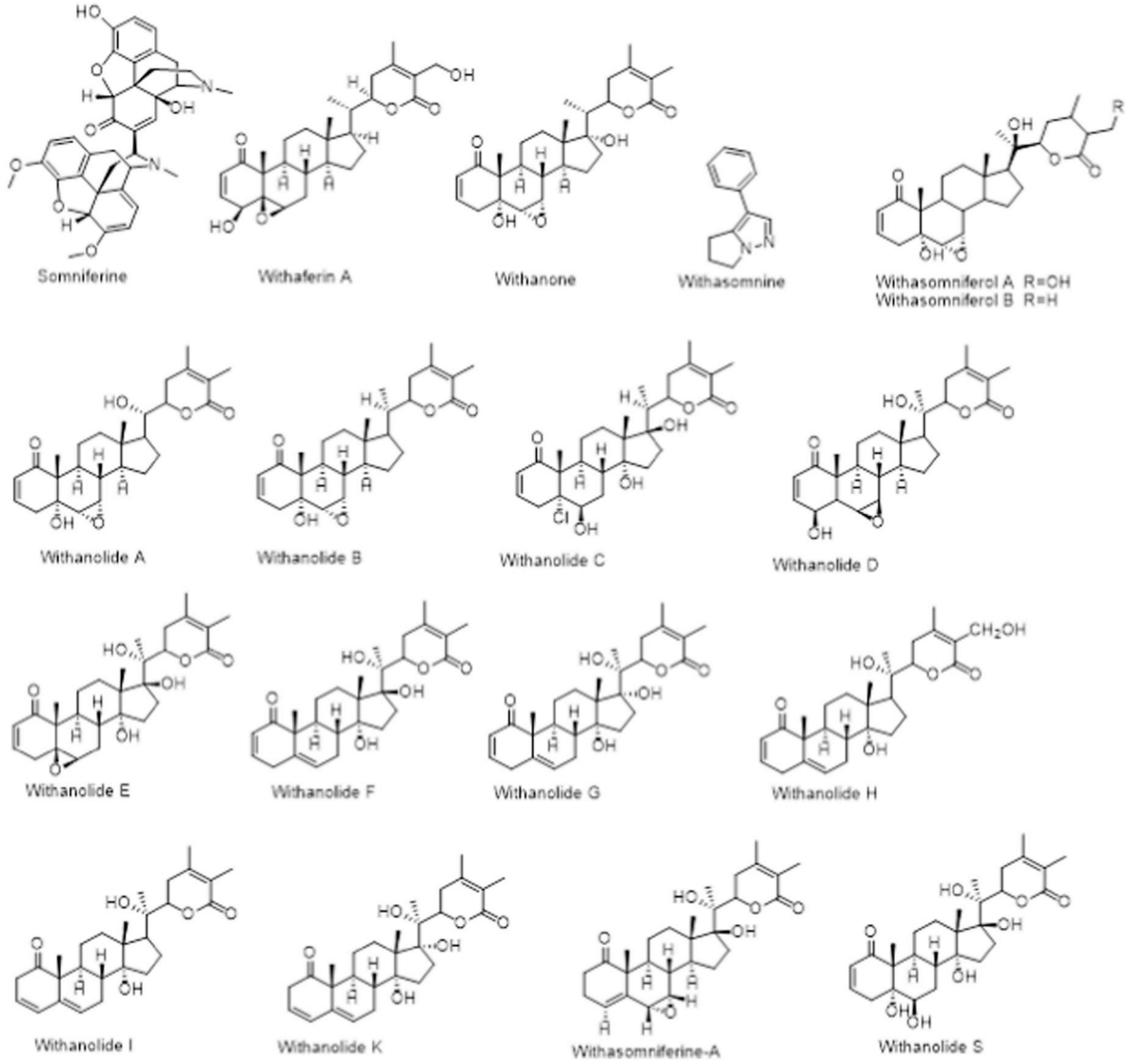

Fig. 1. Major phytoconstituents of Ashwagandha.

science of healing (Shiddamallayya et al., 2016). *Withania sominifera* is known as Asgandnagori (Sharma and Kumar, 2012) or Ba-dzigandha (Scartezzini and Speroni, 2000) in TSM. It is indicated mainly for the treatment of respiratory disorder, hepatic disorders, body strengthening and maintaining hemoglobin level.

Homoeopathy is a form of traditional medicine based on "theory of similarities" which means if the substance causes any symptom in healthy person it will also heal the same symptoms successively in ill person. In this system, only higher dilutions known as tinctures of natural i.e. animal, plants, minerals and synthetic substances are prepared for treating the disease (Shiddamallayya et al., 2016). It is mainly suggested for the treatment of leprosy, nervous disorders, intestinal infection and rheumatism in Homeopathy (Arunachalam, 2002). In 2016, Laidlaw reported the homoeopathic proof of *Withania somnifera* in the thirtieth centesimal potency through double-blind placebo-controlled trial (Laidlaw, 2016).

In China, it is known as Indian ginseng. Currently, Ashwagandha from India is not classified as per Chinese traditional system (CTS) may be due to isolation and distance of countries and their cultures. However, on the basis of comparison between various relevant herbs with Ashwagandha, it can be categorized in CTS as "Tonify Qi" and "Tonify Blood and Essence" (Forman and Kerna, 2018).Though, China on the basis of scientific research and evidences are more successful in promoting the uses of various herbs including *Withania sominifera* (Patwardhan et al., 2005).

In Africa, *Withania somnifera* is the native plant and mainly considered as the weed of contaminated and waste areas. Leaves are used as dressings for infections and inflammations. The root in the form of fine powder mixed with fat of animals (crocodile or python) and is used for sores and abscesses as ointment (Hutchings et al., 1996). It is also used to counteract the foul body odour. The roots are treated and used as enemas for infections of the rectum and pyrexia. It is also used to heal relationships. It is prescribed by African traditional practitioners for fever, cold, bronchial disorder (asthma), venereal infections (syphilis), diarrhea, skin diseases and typhoid. It is also indicated as antihelmintic, antirheumatism, sedative and hypnotics. In the Zulu tradition of Africa, *Withania somnifera* is used to protect people from sorcery. It is also applied as boundaries for insects (Laidlaw, 2016).

4. Common brain disorders

The term brain disorder is not restricted to mean insanity and allied conditions of mental derangement but also includes, to certain extent, the emotional disorders. Often the emotional factors, when cross the state of normalcy, get deranged to become the syndromes of mental disorder. It is stated that the brain has 100 billion neurons and each of

Table 2
Various neuroprotective properties of phytoconstituents of Ashwagandha in preclinical models.

Phytoconstituents	Type of study	Part used for isolation	Dose/IC_{50}	Model/Method	Inference	Reference
Withaferin A (Glutathione conjugate CR-777)	*In-vitro*	Roots	27.1 μM	Mesencephalic neurons exposed to MPP + injury, 6-OHDA injury and α-Synuclein injury	Neuroprotective, suppress oxidative stress (α-Syn aggregation via the induction of the cytoprotective PI3K/mTOR pathway)	Rabhi et al. (2019)
Withaferin A	*In-vitro*	–	2 μM	SH-APP cells(a human neuroblastoma cell line) transfected with HIV-1 Tat (5–100 ng/ml) and coc (0.1–10 μM) to induced neurotoxicity	Reduction in secreted Aβ and induced neurotoxicity in amyloid precursor protein (APP)-plasmid transfected SH-SY5Y cells (SH-APP)	Tiwari et al. (2018)
Withanolide A, withanolide B and withaferin A	*In-vivo*	Roots	5, 10 and 20 mg/kg (intraperitoneally)	Nicotine(1 mg/kg) induced conditioned place preference in male albino mice	Anti-addictive (reversing nicotine induce conditioned place preference)	Dumore et al. (2019)
Withanone	*In-vivo*	Roots	10 and 20 mg/kg	STZ (3 mg/kg) injected intracerebroventricular to male Wistar rats	Protection against oxidative stress and inflammatory cytokines (TNFα, IL-1*β*, IL-6, MCP-1)	Pandey et al. (2018)
Withanone and 27-Hydroxywithanolide B	*In-silico*	–	–	High binding affinity of > -10.5(kcal/mol) against β-secretase 1, mono amine oxidase and phosphodiesterase	Anti-Alzheimer's	Borah et al. (2019)
Withanolide A	*In-vivo*	Roots	10 μmol/kg	Sprague dawley rats were exposed to a simulated altitude of 7600 m (25,000 ft, 282 mm Hg) in a specially designed animal decompression chamber pressure	Reduction in neurodegenration (restoring glutathione depletion) and increase in glutathione biosynthesis through Nrf2 pathway by upregulation of GCLC level in neuronal cells (corticosterone dependent manner)	Baitharu et al. (2014)
	In-vitro	–	5 μg/ml	Glioma C6 (rat) and neuronal IMR32 (human) cell lines against scopolamine (3 mM) induced toxicity	Regulation of neuronal cell markers NF–H, MAP2, PSD-95, GAP-43 and glial cell marker GFAP and with upregulation of DNA damage- γH2AX and oxidative stress- ROS markers.	Konar et al. (2011)
	In vitro	Roots	5 μM	Aβ-induced toxicity in (*Caenorhabditis elegans*) CL4176 worms	Reversed the potential of Aβ followed by acetylcholine and acetyl cholinesterase modulation resulting neuroprotection	Akhoon et al. (2016)
	In-silico	–	–	Ligand interaction with the residues Thr78, Trp81, Ser120 and His442 of human acetyl cholinesterase	Docking simulation results in high binding affinity of the ligand to the receptor	Grover et al. (2012)
	In vitro	Roots	1 μM	Axonal and dendritic atrophy was induced in cultured rat cortical neurons by treatment with amyloid β(25–30)(10 μM)	Regeneration of neuritis, reconstruction of synapses (both pre and post) in neurons	Kuboyama et al. (2005)
	in vivo	Roots	10 μmol/kg/day (orally)	Water maze test (Male ddY mice)	Recovered memory defects	
Withanolides and withanosides	*In vivo*	–	1 g/kg (orally)	APP/PS1 Alzheimer's disease transgenic mice	Reverses Behavioral Deficits and plaque pathology, decreases Aβ levels and promotes, disaggregation of Oligomers, activated microglia proximal to the plaques decrease significantly, enhances LRP and NEP mRNA and down-regulation of hepatic LRP	Sehgal et al. (2012)
Withanolides (IV and V)	Spectrophotometric and *In silico*	–	20.5 and 49.2 μM (acetylcholinesterases) and 29.0 and 85.2 μM (butyrylcholinesterase)	Determination of kinetic parameters, molecular docking, spasmolytic and calcium antagonist activities	Withanolide IV are non-competitive inhibitor of AChE and BChE, Withanolie V are linear mixed-type inhibitor of AChE, High binding affinity for AChE, Exhibits spasmolytic and calcium antagonistic potential	Choudhary et al. (2005)

(*continued on next page*)

Table 2 (*continued*)

Phytoconstituents	Type of study	Part used for isolation	Dose/IC_{50}	Model/Method	Inference	Reference
Withanolide (E, F,J,G,M,N), withafastuosin E and somniferine	*In silico*	–	–	Potential inhibition of gelatinases mainly metalloproteinase i.e. MMP-2 and MMP-9 in comparison with potent gelatinase inhibitors i.e. hydroxamic acid, doxycycline, minocycline, quercetin and reverse hydroxamate)	Inhibition of gelatinases (MMP-9) Therapeutically active in ischemic stroke, hemorrhagic stroke and perinatal hypoxic	Kumar and Patnaik (2018)
Withanolide (aqueous fraction)	*In-vivo*	Roots	12.5, 25, 50 and 100 mg/kg (orally)	Hypoxia time, antifatigue effect, swimming performance time, swimming induced gastric ulceration and hypothermia, immobilization induced gastric ulceration, autoanalgesia	Anti-stress activity in dose dependent manner	Singh et al. (2001)
Withanone	*In-vitro*	–	20 μM	10 μM retinoic induced neuronal distortion on Neuro2A (N2a) mouse neuroblastoma cell line followed by 3-mM NMDA	Neuroprotective effects of Withanone followed by reduction in oxidative stress and pro-apoptotic cytokines	Dar et al. (2017)
Stigmasterol, Withaferin A and withanolide (G and B)	*In silico*	–	–	Inhibition efficiency against poly (ADP-ribose) polymerase-1 (PPAR-1) in comparison to potent inhibitors (FR257517, PJ34 and talazoparib)	High binding affinity for catalytic domain PARP-1 and beneficial in neuro-cytotoxicity and other neurological disorders (like stroke, etc)	Mukherjee et al. (2017)
Glycowithanolides (sitoindoside IX and sitoindoside X)	*In-vivo*	Roots	50–200 mg/kg(orally)	Restraint stress-induced gastric ulcers in albino rats passive avoidance test receiving a foot shock (3 s, 5 mA, DC electric current) in albino rats	Anti-stress and improve memory and learning (Central stimulation characterized by piloerection, increased motility, reactivity to stimuli, spontaneous motor activity and augmented learning acquisition)	Ghosal et al. (1989)
Glycowithanolides	*In vivo*	Roots	20 and 50 mg/kg(orally)	Elevated plus-maze test, Social interaction and feeding latency in an unfamiliar environment	Anti-anxiety (reduced tribulin levels in rat brain) and Antidepressant effect	Bhattacharya et al. (2000)
Withanolide B	*In silico*	–	–	Potent and selective inhibitor of neuronal nitric oxide synthase (nNOS and NOS1) on the basis of selectivity, binding energy and interaction profile	Dual-selective inhibition of both nNOS (iNOS and eNOS) Potential neurotherapeutic agent mainly for disorders mediated by nNOS activation	Kumar et al. (2017)
Withanamides and withanolides	Model system using large unilamellar vesicles	Fruits	1 and 10 μg/ml	Fe^{2+} induced lipid peroxidation assay and $AAPH^{+}$ induced lipid peroxidation assay	Inhibits lipid peroxidation (anti-oxidant)	Jayaprakasam et al. (2004)
Withanamide A and C	*In vitro*	Fruits	50 μg/ml	Rat neuronal cells i.e. PC-12 cells (cell viability assay) and molecular modeling studies (NMR)	Protect PC-12 cells from cell damage induce by β-amyloid Inhibit fibril formation (bind to active site of β-amyloid)	Jayaprakasam et al. (2010)

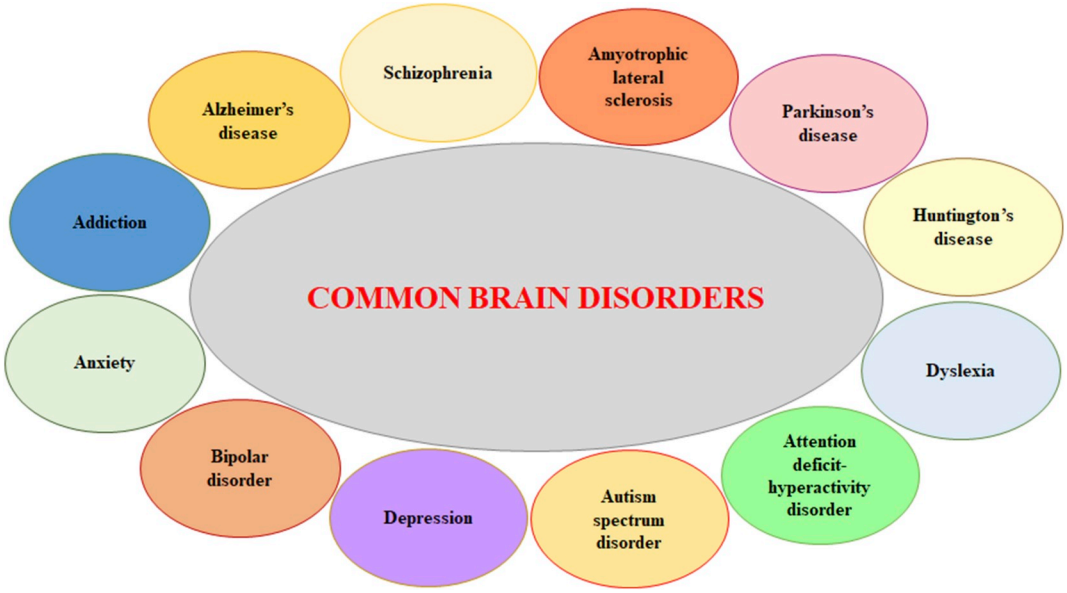

Fig. 2. Common brain disorders.

them connect with many others to form communication networks. These nerve cells have special jobs like thinking, learning, remembering as well as to see, hear, and smell. Brain cells work like tiny factories, receive supplies, generate energy, construct equipment, and get rid of the waste. Brain cells also process and store information to communicate with other cells. The large amount of fuel and oxygen is required for proper functioning and coordination. The common brain disorder is collated in Fig. 2. The information related to different studies conducted on Ashwagandha extracts are summarized in Table 3.

4.1. Ashwagandha in anxiety

Anxiety is defined as 'an emotion characterized by feelings of tension, worried thoughts and physical changes'. Overconsumption of tasty and high-calories food is responsible for chronic positive energy balance where the ingestion of energy exceeds energy expenditure, cause to extra fat in body, weight gain, and obesity (Dinh et al., 2015). The ingestion of high-calorie food has also been associated to various neuropsychiatric disorders like anxiety, mood disorders and binge eating (Castanon et al., 2014).

Powder extract of Ashwagandha leaf studies suggest that it has anti-anxiety, anti-inflammatory, and anti-apoptotic properties which may be recommended to prevent/slow down the adverse effects of obesity and its associated disease (Kaur and Kaur, 2017).

The hydro alcoholic extract of roots of Ashwagandha reported significant anti-stress activity (Singh et al., 2011). Alcoholic extract of Ashwagandha root and seed was given to mice (100 mg/kg intra peritoneal as a single dose) and observed swimming performance. It was observed that the swimming time of Ashwagandha treated mice doubled as compared to normal control. It suggest that Ashwagandha induced a stage of nonspecific increased resistance during stress condition (Mishra et al., 2000; Singh et al., 2010).

Ashwagandha has a significant anti-stress adaptogenic effect as reported by Basic Medical Sciences at Calcutta University. They studied the effect of Ashwagandha on chronic stress in rodents. An electric foot shock received for a period of 21 days, produced hyperglycaemia, glucose intolerance, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression in animals. The extract of Ashwagandha an hour before the shock reduced stress level significantly (Singh et al., 2008). Ashwagandha decreased neuron activity and inhibited nerve cells from over firing. Ashwagandha produces GABA type activity which may indicate it has anti-anxiety activity (Singh et al., 2010).

4.2. Ashwagandha in Alzheimer's

Alzheimer's disease is defined as neurodegenerative disease mainly characterized by progressive memory loss and irreversible decline in cognitive functions. Various *in vitro* and *in vivo* studies highlighted the effect of Ashwagandha and its phytoconstituents in Alzheimer's disorder. Recently, the research was conducted to evaluate the anti-Alzheimer's constituent present in the root extract of Ashwagandha and concluded that Withanone shows significant activity specifically by inhibition of amyloid β-42. Withanone also found to intensify the activity of acetyl choline, glutathione and secretase enzyme (β and γ) and improved the elevation in pro-inflammatory cytokines levels (Pandey et al., 2018). Withanolide-A inhibited human acetyl cholinesterase by high binding affinity, predicted by docking simulation studies (Grover et al., 2012).

In experimental studies performed by Sehgal et al., semi purified root extract of Ashwagandha mainly containing withanolides and withanosides, which reversed Alzheimer's disease by producing neuroprotective effects against H_2O_2- and β- Amyloid cytotoxicity in APP/PS1 transgenic mice and APPSwlnd mice (line J20) of Alzheimer's disease by up-regulation of lipoprotein receptor-related protein in liver (Sehgal et al., 2012).

Ashwagandha at dose of 100 mg/kg shows enervation of AChE inhibition and cognitive impairment thus showing protective effects against propoxur exposure in rats (Yadav et al., 2010). Methanolic chloroform extract of Ashwagandha elicits high cell viability and enhances PPAR-c levels along with restoration in morphology of cell against SK-N-MC cell line infected by β-amyloid (Kurapati et al., 2013). Furthermore, aqueous extract of Ashwagandha containing Withanolide derivatives including withaferin A shows potential protection in differentiated pheochromocytoma PC12 cells infected by hydrogen peroxide and Aβ cytotoxicity (Kumar et al., 2010).

4.3. Ashwagandha in Parkinson's

Parkinson's disease is a progressive, age-dependent neurodegenerative disorder primarily characterized by depletion of dopaminergic substantia nigra neurons caused by genetic and environmental factors. It is associated with oxidative stress, mitochondrial dysfunction and abnormality in aggregation of protein. Ashwagandha have been

Table 3
Reported studies of Ashwagandha extracts including dose, mechanism and method employed to highlight its role in brain related disorders.

Extract	Dose	Mechanism involved	Method	Inference	Reference
Ethanolic root extract of Ashwagandha	100 mg/kg(orally)	Improvement in impaired spatial memory and oxidative stress (modulation of MDA levels, ROS, SOD and catalase) Significant increase in NMDA receptor expression	Bisphenol A (50 μg/kg) injected orally for 21 days to male Swiss albino mice	Neuroprotective and anti-oxidant (improves cognitive impairment)	Birla et al. (2019)
Aqueous leaves extract of Ashwagandha	140 mg/kg(orally)	Improvement in locomotor coordination, working memory and learning functions Normalization in expression of synaptic plasticity markers Restoration of signaling proteins (BDNF-TrkB, PLCγ, IP3R and PI3K, Akt)	LPS (5 mg/kg) injected intraperitoneally to male Wistar albino rats for 8 weeks Behavioural studies (rotarod test, narrow beam walk test, novel object recognition test)	Attenuation of neuroinflammation and neurodegeneration	Gupta and Kaur (2019)
Aqueous suspension of Ashwagandha	100 mg/kg(orally)	Improvement in acquisitions and retension in step-down latency test Reverse oxidative stress by attenuating effect of dieldrin on MDA level, protein carbonyl and reduced glutathione activity	Dieldrin 5 mg/kg-induced modulation of cognitive function and oxidative stress in male Wistar rat	Neuroprotective and anti-oxidant	Ghosh et al. (2019)
Root extract of Ashwagandha	0.75 and 1.00 mg/ml (orally)	Regulation of opioid receptors (Mu-opioid (MOP) and nociceptin (NOP)) gene expression in SH-SY5Y cells	10 μM morphine, 100 μM naloxone induced toxicity on human SH-SY5Y neuroblastoma cells	Prevention of morphine-elicited gene expression alteration Modulation of MOP and NOP expression	Caputi et al. (2018)
Aqueous extract of Ashwagandha	200 mg/kg(orally)	Inhibited the caspase independent mechanism of apoptosis by the inhibition of poly (ADP-ribose) polymerase-1 and improve anti-oxidant enzyme hemoxygenase-1(HO-1)	Permanent middle cerebral artery occlusion (pMCAO)	Neuroprotective effect	Raghavan and Shah (2015)
Ashwagandha aqueous suspension	100 mg/kg(orally)	Attenuation of AChE inhibition & improvement in cognitive impairement	Propoxur at dose of 10 mg/kg dissolved in olive oil administered to wistar albino rats	Anti-Alzheimer's	Yadav et al. (2010)
Methanol: Chloroform (3:1) extract of ashwagandha	–	Neutralization of toxic effects (supported by MTT cell viability assays and the peroxisome proliferator-activated receptor-γ (PPARγ) levels)	β-amyloid induced toxicity and HIV–1_{Ba-L} (clade B) infection using a human neuronal SK-N-MC cell line	Anti-Alzheimer's	Kurapati et al. (2013)
Ethanolic extract of Ashwagandha	40 mg/kg(orally)	Improvement in oxidative stress profile & restoration of behavioral performance	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson-like symptoms in Balb/c mice	Anti- Parkinson's	Bhatnagar et al. (2017)
Ethanolic extract of Ashwagandha	100 mg/kg(orally)	Improvement in the motor movement patterns and gripping ability, restoration of tyrosine hydroxylase (TH) immunostaining in substantia nigra and modulation of apoptotic mechanism thus improvement in behavioural performance	Maneb (30 mg/kg) and Paraquat (10 mg/kg) induce Parkinsonism in swiss albino mice	Anti- Parkinson's	Prakash et al. (2013); Prakash et al. (2014)
Ashwagandha root extract	(0–0.05%) Enriched diet	Improvement in locomotor function, modulation of oxidative stress and normalize of levels of succinate dehydrogenase, MTT, membrane bound enzymes viz., NADH-cytochrome-c reductase and succinate cytochrome-c reductase	Rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in *Drosophila melanogaster*	Neuromodulator and Anti-Parkinson's	Manjunath and Muralidhara, 2015
Ashwagandha root extract	100 mg/kg(orally)	Increase in levels of dopamine, 3,4-dihydroxy-phenylacetic acid and homovanillic acid & normalization of lipid peroxidation marker	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at dose of 20 mg/kg to male Swiss albino mice	Anti- Parkinson's	Rajasankar et al., 2009a
Ashwagandha leaves extract	100 mg/kg(orally)	Improvement in posture and the coordinated motor skills including voluntary movements, walking, and reduced stride length	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at dose of 20 mg/kg to male Swiss albino mice	Anti- Parkinson's	Rajasankar et al. (2009) b
Ashwagandha root extract	100 mg/kg(orally)	Improvement in the mice's behaviour & normalization in superoxide dismutase and catalase	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at dose of 20 mg/kg to male Swiss albino mice	Anti- Parkinson	RajaSankar et al., 2007

(*continued on next page*)

Table 3 (*continued*)

Extract	Dose	Mechanism involved	Method	Inference	Reference
Ashwagandha root extract	100 and 200 mg/kg (orally)	Normalization of lipid peroxidation and nitrite and lactate dehydrogenase enzyme levels Inhibit blockade in ATP synthesis by inhibiting the mitochondrial complex activity in the different regions (striatum and cortex)	3-Nitropropionic acid 10 mg/kg for 14 days Intraperitoneal to rat	Anti-Huntington's disease	Kumar and Kumar (2009)
Ashwagandha root extract	100 and 200 mg/kg (orally)	Improvement in cognitive impairment (in Morris water and plus maze tests) and restored GSH, total glutathione, oxidized glutathione, GST and attenuated acetyl cholinesterase levels	3-Nitropropionic acid 10 mg/kg for 14 days Intraperitoneal to rat	Neuromodulation	Kumar and Kumar (2008)
Ashwagandha ghrutha (fat extract)	20 and 40 mg/kg(orally)	Inhibition of immobility time Antagonism of ptosis, catatonia and sedation	Forced-swim test and tail-suspension test Reserpine induced ptosis, catatonia and sedation	Anti-depressants	Jayanthi (2012)
Pure powder of Ashwagandha	50,100 and 150 mg/kg (orally)	Increase in avoidance response, escape failure and immobility period	Albino rats subjected to electric shock in learned helplessness test and swimming in forced swimming test	Anti-depressants	Maity et al. (2011)
Aqueous extract of Ashwagandha root	100 and 200 mg/kg (orally)	Improved behavioral alterations, altered oxidative stress markers and restored histo-architecture of cerebellum	Sodium valproate (400 mg/kg subcutaneous) in BALB/c mice	Anti-autism	Veeresh et al. (2016)
Methanolic extract of Ashwagandha root	75 mg/kg(orally)	Prevention in both morphine- and ethanol-elicited increases of dopamine in the shell of the nucleus accumbens	Morphine- and ethanol induce increase in dopamine in male Sprague dawley rat	Motivated behavioral alterations	Bassareo et al. (2019)
Ashwagandha extract	50,100,200 mg/kg (intraperitoneal)	Reversing nicotine induce conditioned place preference	Nicotine(1 mg/kg) induced conditioned place preference in male albino mice	Anti-addictive	Dumore et al. (2019)
Ashwagandha extract	5 mg in 200 μL sterile buffered saline	Reduction in levels of misfolded SOD1 and of phosphorylated NFκB	Transgenic C57BL/6 mice carrying G93A sod1 mutant	Anti-amyotrophic lateral sclerosis	Dutta et al. (2018)
Ashwagandha root extract	100–300 mg/kg(orally)	Amelioration of vacuous chewing movements and tongue protrusions, reversed the decrease in forebrain SOD	Haloperidol (1.0 mg/kg i.p.) given chronically to male Wistar rats to induce oral dyskinesias	Anti-oxidant Inhibit neurodegeneration	Naidu et al. (2003)
Ashwagandha root extract	50, 100 and 200 mg/kg (orally)	Improvement in disruption of acquisition and retention Reduced the latency to reach the shock free zone Improved memory consolidation	Scopolamine (0.3 mg/kg)- administered intraperitoneal to Albino mice Acute treatment with electroconvulsive shock, immediately after training Chronic treatment with electroconvulsive shock for 6 successive days at 24 h intervals	Nootropic effect and anti-amnesia	Dhuley (2001)

explored in various studies to ameliorate Parkinson's disorder. Ashwagandha ethanolic root extract has been found to reverse the Parkinson-like symptoms in MPTP induced Parkinson in Balb/c mice (Bhatnagar et al., 2017). Ashwagandha conferred impaired cholinergic function and depletion in dopamine levels in rotenone model of *Drosophila melanogaster* by suppression of oxidative stress and mitochondrial dysfunctions (Manjunath and Muralidhara, 2015). Mouse, when treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine showed alteration in catecholamines, antioxidants and lipid peroxidation marker level. Treatment with 100 mg/kg of Ashwagandha leads to normalizing dopamine, 3,4-dihydroxy-phenylacetic acid, homovanillic acid, antioxidants (glutathione and glutathione peroxidise) and thiobarbituric acid reactive substance levels in striatum and improvement in motor functions (RajaSankar et al., 2009a,b).

Another study conducted in 2007 indicated improvement in mice behaviour, antioxidant status and lipid peroxidation levels in the brain of Parkinson's induced mice (RajaSankar et al., 2007). Prakash et al., in 2013 demonstrated through *in vivo* research that Ashwagandha root extract in Maneb-Paraquat (MB-PQ) induced Parkinsonian mouse model showed neuro-ameliorative effect by improving motor movements and gripping ability. Further, showed decline in catalase levels and rise in nitrite and lipid peroxidation levels, thus, counterbalancing pro-oxidants and oxidative stress in Parkinson's disease (Prakash et al., 2013). In 2014, a study showed alleviation of Parkinson's by ethanolic root extract of Ashwagandha, majorly by inhibition of apoptotic pathway and oxidative stress in dopaminergic neurons. Significant improvement in oxidative stress and apoptotic state is due to the reduced expression of iNOS and Bax and inducing Bcl-2 protein expression in MB-PQ induced Parkinsonian mouse model (Prakash et al., 2014). Hence these data suggests the neuromodulatory effect of Ashwagandha. Whereas further studies are needed to assess the precise mechanism to support the clinical use of the plant as an anti-parkinsonian drug.

4.4. Ashwagandha in schizophrenia

Schizophrenia is defined as a chronic psychiatric disease associated with perturbations in thinking, perception, behaviour and other cognition characteristics. It also affects language and causes hallucinations, delusion and other psychotic illness (Wei et al., 2018). Variety of preclinical and clinical studies concluded the role of Ashwagandha in treating schizophrenia. Neuroleptics are widely used in the therapy of schizophrenia. In haloperidol induced orofacial dyskinesia, Ashwagandha root extract at dose of 100–300 mg/kg, dose dependently ameliorates vacuous chewing movements and tongue protrusions superiorly by reduction in lipid peroxidation and increase in forebrain SOD and catalase levels with no effect on glutathione levels. Thus, Ashwagandha may be efficient in suppressing extrapyramidal neuroleptic-induced effects (Naidu et al., 2003).

A randomized, double-blind, placebo-controlled study highlighted the role of standardized extract of Ashwagandha as adjunct to treat Schizophrenia symptoms. The extract exhibiting anti-inflammatory and immunomodulation properties result in improving psychopathology and stress symptoms in schizophrenia patients (Chengappa et al., 2013). In 2016, Kumar and colleagues conducted *in silico* studies to explore potential of various phytochemicals of Ashwagandha as neuroprotective by inhibiting GluN2B-containing NMDA receptor. Further, it proved that GluN2B-containing NMDA hastens neurodegeneration in schizophrenia. Thus, phytochemicals in Ashwagandha are found to be potential antagonist for NMDA receptor and may be used for treatment of various neurodegenerative conditions (Kumar and Patnaik, 2016).

4.5. Ashwagandha in Huntington's disease

Huntington's disease is a progressive neurodegenerative disorder characterized by destruction of basal ganglia neurons and oxidative stress. It is an autosomal dominant inherited syndrome associated with disturbance in muscle function, emotional disturbance, and dementia. It also includes lack of focus, concentration, depression, short-term memory loss, language, speech and movement problems (Manoharan et al., 2016). Multiple lines of *in vivo* and clinical evidences implicated Ashwagandha in treatment of Huntington's disorder. Nitropropionic acid induction mimics the abnormalities similar to Huntington's disorder particularly oxidative stress, gait abnormalities and mitochondrial dysfunction. Inhibition of complex II further causes behavioural, enzymatic and biochemical alterations. Ashwagandha root extract at dose of 100 and 200 mg/kg, dose dependently improved various alterations and shows neuroprotective potential dominantly by antioxidant activity (Kumar and Kumar, 2009). Further, the involvement of GABAergic system in Huntington's disease pathogensis has been reported. Ashwagandha acts by GABAergic system and antioxidant potential restores acetyl cholinesterase and glutathione enzyme level and improves cognitive function thus making it suitable candidate for the treatment of Huntington's disorder (Kumar and Kumar, 2008).

4.6. Ashwagandha in dyslexia

Dyslexia is the brain associated disorder primarily characterized by learning disability, difficulty in reading and spelling words accurately and fluently. Various other symptoms such as impairment in memory, speech, visual and motor control are also associated with dyslexia (Bull, 2007). Phonological coding defects i.e. failure to speak and get the sound of a word, lacking phonemic division i.e. powerlessness to break a word into part sounds, poor vocabulary advancement, and inconvenience in segregating linguistic and syntactic contrasts among words and sounds are common key symptoms of dyslexia (Lyon et al., 2003). Different clinical and exploratory research in reference to Ashwagandha directly or indirectly gave confirmations in regard to progress in cognitive functions which might be useful in improving the inability in dyslexia (Sharma et al., 2012). In 2001, Dhuley reported improvement in memory-consolidated induced by electroconvulsive shock in mice receiving Ashwagandha root extract (Dhuley, 2001).

4.7. Ashwagandha in depression

Depression is a heterogeneous disorder associated with brain health mainly mood swings and thoughts, misbehaviour, disappointments, sadness, hopelessness and loss of physical activities and self-worth. In addition depression is accompanied with disturbance in appetite, sleep pattern and other daily work along with symptoms of anxiety (Ahmed et al., 2017). Experimental studies have reported the ameliorative effect of Ashwagandha in depression.

In 2000, Bhattacharya et al., isolated the bioactive compound glycowithanolides from Ashwagandha roots and investigated its potential as antidepressant at the dose of 20 and 50 mg/kg. Glycowithanolides were found to exhibit comparable antidepressant effect to imipramine in behavioural despair and learned helplessness induced by forced swim tests Thereby, supporting the use of Ashwagandha as mood stabilizer (Bhattacharya et al., 2000).

Reports on Ayurvedic formulations containing Ashwagandha, “Ashwagandha ghrutha (fat extract), supported its potential as antidepressant by significantly reducing immobility time in forced swim test induced behavioural despair, tail suspension test and reserpine antagonism in anti-reserpine test at a dose of 40 mg/kg (Jayanthi, 2012). Another study evaluated the role of Ashwagandha in depression through learned helplessness and forced swimming test. It is also reported enhanced anti-depressant efficacy by combining Ashwagandha with imipramine in animal model (Maity et al., 2011).

4.8. Ashwagandha in autism

Autism is the brain inflammatory disorder associated with

disabilities of learning and social activity. It is characterized by oxidative stress, astrocytes and microglia activation, alteration in pro-inflammatory cytokines, 8-oxo-guanosine and neuronal depression. It also includes high food intolerance, anxiety, increasing auto-antibodies of anti-brain protein, lowering levels of reduced glutathione, sulfation and methylation (Rasool et al., 2014). Recent report indicated the potential of Ashwagandha in sodium valproate induce autism in rodent model by improvement in altered behavioural and oxidative stress. Restoration in number of purkinje fibres, neuronal degeneration and chromatolysis in histo-architecture studies of cerebellum further confirms its ameliorative effect in autism (Veeresh et al., 2016).

4.9. Ashwagandha in addiction

Addiction is defined as complex, chronic brain disorder associated with physical and psychological dependence on chemical, drug or activity. It is characterized by alterations in behaviour, thinking, body functions, learning, memory and decision making (Everitt and Robbins, 2016). It mainly includes use of alcohol, cocaine, nicotine, marijuana, opioid, caffeine, inhalants or activity like gambling. Transduction and transcription factors are found to be associated in developing and maintaining addiction in molecular studies (Koob and Volkow, 2016).

In a recent study, it is reported that Ashwagandha inhibits the stimulation of neuron circulation and dopamine transmission specifically in ventral tegmental region of dopaminergic neurons and in the nucleus accumbens shell thus preventing the alteration of behaviour and biochemical changes caused due to electrochemical and neurochemical modifications induced by morphine and ethanol (Bassareo et al., 2019).

Another study investigated the ameliorative effect of Ashwagandha extract in preventing nicotine attributed addiction. It was concluded that Ashwagandha attenuates nicotine induced place preference condition mice, thus highlighting anti-addictive potential due to modulation of nicotine cholinergic receptor (Dumore et al., 2019).

Recently, a patent has also been filed and published with publication number US 2019/0038700 A1 which enlisted various medicinal plants imparting anti-smoking potential with Ashwagandha as one of them. Furthermore, the synergistic potential of Ashwagandha with various other plants in formulating nicotine free anti-smoking formulation has also been reported (Tamoli, 2019).

Another study reported that in chronic alcohol exposure Ashwagandha churna causes reduction in both withdrawal anxiety and intake of ethanol. Dominantly, the mechanism involved in anti-addiction is via modulation of GABAergic and serotonergic system (Bansal and Banerjee, 2016). In view of several above studies, Ashwagandha is found to be effective in reversing addiction.

4.10. Ashwagandha in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis also known as frontotemporal lobar degeneration is multi-factorial neurodegenerative disorder affecting neurons involved in motor activities and voluntary muscles. It basically includes alteration of upper and lower motor neurons in cerebral cortex and latter in medulla and anterior horn of spinal cord. Failure in upper motor neurons result in muscle stiffness and spasticity whereas lower motor neurons lead to muscle twitching further leading to degeneration and loss in connectivity in synapse thus resulting atrophy (Brown and Al-Chalabi, 2017). It is found to initiate the focal weakness which further escalates to muscles damage including respiratory muscles. Ashwagandha in $SOD1^{G93A}$ mice was found to slow down the disease progression, improved motor performance and increased the number of motor neurons in lumbar spinal cord due to induction of autophagy activity. Additionally, the levels of misfolded SOD1 were diminished and phosphorylation of NF-κB was prevented (Dutta et al., 2018).

4.11. Ashwagandha in attention deficit hyperactivity disorder

Attention deficit hyperactivity disorder (ADHD) is a heterogeneous brain disorder associated with distractibility or inattention, may or may not accompany with hyperactivity. It is more common in children and often seems to begin in childhood and may continue further to adulthood, maximally occurs in males than in females. It mainly interferes with functioning and development by alteration and interruption of focus, tasks, thinking, desire, gratification, talks, attention, decision, behaviour, etc (Barkley, 2015). It co-occurs with number of brain disorders including mood, anxiety and conduct disorders (Biederman et al., 2001). It is characterized by alteration in dopamine and norepinephrine reuptake thus medications which blocks dopaminergic and noradrenergic activity inhibits ADHD (Biederman, 2005).

Nootropic effects of various herbs including Ashwagandha plays an important role in treating and preventing ADHD. Multiple clinical trials and animal studies have reported its use in anxiety, cognitive and neurological disorders (Bhattacharya et al., 2000). Kanyaiyaa and colleagues, reported immunomodulatory and CNS effects of new glycowithanolides mainly sitoindoside IX and sitoindoside X, at dose of 50–200 mg/kg (Kanyaiyaa et al., 2014). Thus, Ashwagandha was found to be beneficial in ADHD due to its immunomodulatory and CNS effects mainly in stress, memory and learning. However further studies including both animal and clinical trials are required to reveal the mechanistic pathway of Ashwagandha in ADHD.

4.12. Ashwagandha in bipolar disorder

Bipolar disorder also known as manic depression is a brain disorder that effects mental health by causing extreme swings in mood mainly depression and mania or hypomania (Belmaker, 2004). It is associated with sadness, hopelessness, low interest, lack of pleasure, sleeplessness, high energy, judgemental, behaviour alteration, euphoric, high energy, irritability which is either emotional highs i.e. mania or hypomania or lows i.e. depression. Hallucination, anxiety, insomnia, ADHD, post-traumatic stress and other symptoms may co-occur with bipolar disorder (Grande et al., 2016). Till date, one study has been conducted to report the role of Ashwagandha in bipolar disorder. Cognitive dysfunction leads to alteration in functional recovery in bipolar disorder. Randomized placebo-controlled study reported the efficacy of Ashwagandha at dose of 500 mg/day as adjunct in bipolar disorder. Ashwagandha was found to be significantly beneficial as compared to placebo in cognitive tasks (Chengappa et al., 2013).

Probable mechanism involved in above mentioned various brain disorders by reported active constituents of Ashwagandha is summarized in Fig. 3.

5. Safety assessment studies of Ashwagandha

Numerous studies have been conducted by various researchers to evaluate the toxic effects of Ashwagandha. It is considered as healthy, safe and dependable drug. Subacute toxicity studies was conducted by Aphale and colleagues on combination of Ashwagandha and Ginseng for 90 days in three doses and found to increase body weight and liver weight but improvement in hematological parameters was observed. Histopathological and gross evaluation of brain, kidney, heart, liver, spleen, kidney, testis and ovaries was found to be normal. Thus, the study revealed no toxic effects in rats (Aphale et al., 1998).

In another study, alcoholic extract of Ashwagandha root was screened for its acute toxicity in Swiss albino mice and subacute toxicity in Wistar rats at a dose of 1100 mg/kg administered intraperitoneally. No mortality was found at the therapeutic dose, however increment of dose to 1260 mg/kg led to death. Thus, LD_{50} was found to be 1260 mg/kg body weight. In subacute toxicity, 100 mg/kg dose of extract was administered for 30 days and no changes were reported in blood parameters. However, a decrease in spleen, thymus and adrenal weights

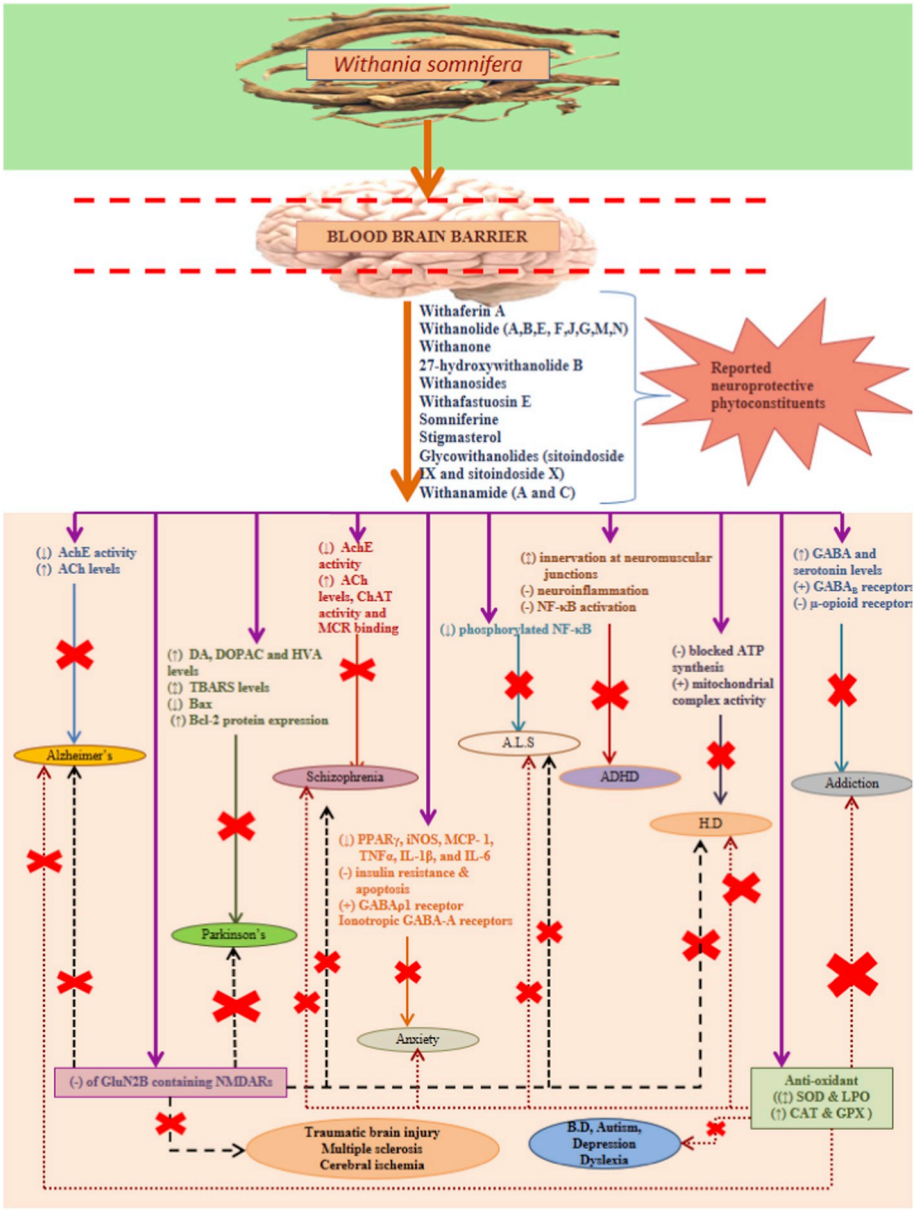

Fig. 3. Summary of proposed mechanism involved in multiple brain disorder by active constituents of Ashwagandha root.

in male rats and an increase in acid phosphatase content was observed, but other biochemical parameters were found to be in normal ranges, in both sexes (Sharada et al., 1993).

In 2012, a study was conducted to evaluate the toxic effects of hydroalcoholic root extract of Ashwagandha. Root extract was administered to rats at dose of 2000 mg/kg orally once and observed for 14 days. No toxic effects and mortality was reported in acute toxicity study. Further, in subacute toxicity study, rats were administered the extract at dose of 500, 1000 and 2000 mg/kg. After 28 days, no changes were observed in body and various organs weight. No dose related changes were found in haematological and biochemical parameters and no treatment related histopathological lesions were noted. Thus, the study claimed no toxic effects of hydroalcoholic root extract of Ashwagandha at dose level of 2000 mg/kg body weight per day in rats (Prabu et al., 2013).

Recently, a study was conducted to evaluate the chronic toxic effects of Ashwagandharishta on kidney functions. The study included administration of Ashwagandharishta to both male and female albino rats for 51 days and observed change in serum urea, uric acid and creatinine levels. In male rats, serum levels of urea and uric acid did not show any significant change at all three doses i.e. low, medium and high dose, but serum creatinine levels were found to be increased in medium dose level group. However, in female rats, no significant changes were observed in levels of serum urea, uric acid and creatinine in respect to low, medium and high dose levels (Rahman et al., 2019).

Another recent study was conducted by Kumar et al., highlights the acute and subacute toxicity studies on patented poly herbal formulation which contains roots of Ashwagandha as one of the ingredient. No mortality was observed in acute toxicity study. In subacute toxicity studies, daily food intake, water intake, biochemical, hematological and histopathological parameter failed to exhibit any significant change. Also no major changes in biochemical enzymes including liver and renal enzymes mainly SGOT, SGPT, ALP, LDH, GGT and ACP were observed (Kumar et al., 2019). Thus, from the above studies, Ashwagandha is found to be safe and may further be explored for therapeutic utilization. As, no toxicity was observed in acute, subacute and chronic toxicity studies which determines the wide safety margin of Ashwagandha.

Table 4
List of patents published from 2010 to 2019 on Ashwagandha related to brain disorders.

Patent no.	Publication date	Title	Activity
JP2013001666A	07-01-2013	Nutrient composition	Anti-oxidant
US8636985B2	28-01-2014	Functional formulation in chewing gum	Reduction of stress and/or enhancement of energy and mental clarity
US20150071993A1	12-03-2015	Compositions and Methods for Improving Sleep Using A Nutraceutical Formulation	Maintains sleep as well as alleviate pain to improve sleep
US20150320814A1	12-11-2015	Nutraceutical formulation for treatment of anxiety and depression	Anti-anxiety and anti-depression
AU2013299656B2	22-12-2016	Multi-component formulation for improving neurological function	Treatment of neurological and neuropsychiatric diseases
CA3002624A1	27-04-2017	A process to enhance the bioactivity of ashwagandha extracts	Immunomodulatory activity, anti-inflammatory activity, anti-stress activity, antidiabetic activity and sleep quality
EP2776462B1	10-01-2018	Indolealkylamino-withasteroid conjugates and method of use	Anti- Alzheimer's, ant-anxiety and anti-depressive disorders
WO2018027127A1	08-02-2018	Compositions for mental alertness and methods of making and using thereof	Mental alertness
US20180042979A1	15-02-2018	Use of a withania extract for the treatment of amyloid-related diseases	Amyloid-related diseases, including Alzheimer disease.
US20180042980A1	15-02-2018	Multifunctional formulation comprised of natural ingredients and method of preparation/manufacturing thereof	Anti-Alzheimer's, and multiple sclerosis
WO2019038454A1	18-02-2018	Functional chewing gum comprising phytonutrients and adaptogenic herbs	Boost mental clarity and concentration
JP2018511619A	26-04-2018	Use of Ashwagandha extract for the treatment of α-synucleinopathies	α-synucleinopathies including Parkinson's disease
KR101971574B1	23-07-2018	Cosmetic composition for improving sleep disorder or maintaining deep sleep comprising withania somnifera, Morus australis and pure tea extracts	Chronic sleep disorders
EP3359177A1	15-08-2018	Use of a withania extract for the treatment of demyelinating diseases	Multiple sclerosis disease
WO2018156202A1	30-08-2018	Anti-psychotic composition and treatment methods	Anti-schizophrenia or schizoaffective disorder
CN106008641A	06-11-2018	Withania somnifera lactide compound, method for extracting same and application of withania somnifera lactide compound	Anti-inflammatory
US20180339008A1	29-11-2018	Natural product compositions for treating or managing symptoms of add, ADHD, anxiety, and depression	Treatment of addiction, ADHD, anxiety and depression
EP3074414B1	9-01-2019	Withanolides useful for the treatment of neurodegenerative diseases	Treatment of neurodegenerative diseases
US10183053B1	22-01-2019	Multi-component formulations	Treatment of cognitive decline, Alzheimer's disease, and other dementias.
US20190038700A1	07-02-2019	Nicotine free herbal composition for smoking de-addiction and treatment of side-effects and/or ailments from smoking	Anti-smoking
EP3442553A1	20-02-2019	Use of a withania extract for the treatment of neuromuscular diseases	motor neuron diseases like amyotrophic lateral sclerosis
US10213507B2	26-02-2019	Compositions and methods for treating HIV-associated neurocognitive disorders	Anti-addiction

6. Recent patents of Ashwagandha in brain disorders

Currently, patents are considered as the most significant and reliable source of information. Patents contain information in all depths and breaths and is estimated that 70–75% information are not available anywhere else.

Various patents on Ashwagandha are available either alone or in combinations with other medicinal plants for use in various diseases includes antimicrobial, anti-lipidemic, anti-diabetic, anti-pyretic, anti-inflammatory, anti-ageing, anti-HIV, antiarthritic, anti-bacteria, anti-microbial, anti-hyperlipidemic, anti-cancer, brain disorders, etc. Recent patents in period from 2010 to 2019 have been enlisted in Table 4.

7. Discussion & conclusion

Review is an important tool to summarize the evidences relating to effectiveness of health care interventions accurately and reliably. In this review, we evaluated the effects of Ashwagandha on brain related disorders. Ashwagandha and its extract have been used since decades to treat various diorders. Ashwagandha is largely used as an immunomodulator in recent times and indicated anti-diabetic, hepatoprotective, anticancer, anti-inflammatory activity as well. Ashwagandha has gained interest from the last three decade for its potential in brain related disorders. Ashwagandha is chemically enriched with numerous active constituents such as withanolides, sitoindosides and several valuable alkaloids that have been explored through valid scientific analysis. These constituents have been documented to be useful for curing brain related disorder. Several studies are being conducted worldwide to explore the pharmacological benefits of the plant and its constituents.

In India, Ashwagandha is used as a household remedy, and is considered as the best memory enhancing tonic for the elderly and children. It is one of the best nervine tonics in Ayurveda, the most ancient system of medical sciences (Singh et al., 2008). Various publications on Ashwagandha have reported its effectiveness for brain related disorders. It has been perceived that most of the published data are based on *in vitro* and *in vivo* studies and few clinical data is available to support its evidence of activities in multiple diseases. There is no direct study to compare the effect of Ashwagandha and its extract with established, approved drugs in a given indication. Thus, Ashwagandha and its extract must be considered as a dietary supplement, not as a drug.

The reported scientific data supports that Ashwagandha is a real potent regenerative tonic due to its multiple pharmacological actions like anti-stress and neuroprotective. It is useful for different types of neurodegenerative diseases such as Parkinson's, Huntington's and Alzheimer's diseases.

The mechanism responsible for Ashwagandha as neuroprotective to the larger degree is due to its antioxidant activity. Antioxidant activity is considerably due to a decrease or normalization in reversed lipid peroxidation levels (Bhattacharya et al., 2000) and increase or normalization of elevated superoxide dismutase levels (mainly in cerebellum, striatum, hippocampus, frontal cortex, etc) which must be followed by activity of enzymes catalase (Prakash et al., 2013) and glutathione peroxidise (Gupta et al., 2003) in striatum, cortex, hippocampus and other parts of brain. Ashwagandha also regulates non-enzymatic antioxidant levels by restoring the levels of glutathione which is an endogenous member of antioxidant defence system (Maher, 2005). Glutathione acts by reacting with oxygen free radicals, organic peroxides and also regulate enzymes (glutathione peroxidise and glutathione-S-transferase) by acting as substrate (RajaSankar et al., 2009a,b). It promotes formation of dendrites through GABA mimetic

effect. Ashwagandha and its extracts improve energy levels and mitochondrial health so it is effective as an anxiolytic (Singh et al., 2011).

Dopaminergic activity of a drug can enhance memory, and number of papers are published showing dopaminergic activity of Ashwagandha root (Barch, 2004; Prakash et al., 2014). A number of *in vivo* and clinical studies have proved the nootropic activity of Ashwagandha and its extract (Dhuley, 2001; Chengappa et al., 2013; Pingali et al., 2014). Neuroprotective effects of Ashwagandha supports its efficacy in Alzeimer's patients (Kuboyama et al., 2014; Ven Murthy et al., 2010).

Ashwagandha and its extract have anti-stress and anxiolytic effect and these effect are involved in memory enhancing activity and used when normal mental activities of human are affected (Eysenck et al., 2007). Beside the Ashwagandha root, its leaf extract indicated probable effect on antioxidant induction in the Parkinson's disease mouse brain (RajaSankar et al., 2009a,b). The dopamine plays an important character in motor control. The decrease levels of catecholamines and oxidative stress are the important reasons of neurodegeneration in Parkinson's disease and causes loss of motor function seen in Parkinson's disease patients (Mishra et al., 2000; Halliwell, 2001). The increased levels of catecholamine after Ashwagandha treatment suggests that Ashwagandha induces catecholamines in the Parkinson's disease.

Thus beside antioxidant various other mechanisms are considered to be responsible for protective effect of Ashwagandha in various CNS disorders including inhibition of acetyl cholinesterase activity, increase in acetylcholine receptor expression (anti-Alzheimer), increase expression of acetylcholine receptor (Cognitive impairment) and dopamine receptor (Parkinson's disease). Further, it also causes inhibition in glucocorticoids and increase in serotonin concentration (stress and other related disease), protection of mitochondria (Huntington's disease) and increase in cyclic adenosine monophosphate (Cerebral ischaemia) (Durg et al., 2015). Ashwagandha is chemically enriched with numerous active constituent's mainly steroidal alkaloids along with lactones which are responsible for its various neuroprotective role in CNS disorder.

A proper, well-documented pharmacological activity of the phytochemical composition of Ashwagandha is lacking. Various individual constituents have been isolated and characterised for their brain related disorders, but still need to discover and explore the treasure to identify the complete bioactive constituents in this plant. This would certainly help to promote an extensive use of Ashwagandha and its extract and the design of new product for various brain disorder. The above findings clearly indicate that the use of Ashwagandha and its constituents has a reasonable and scientific basis. Ashwagandha and its extract showed countless potential as safe and active candidate in brain related disorders. More robust scientific evidence in relation to pharmacological evaluation including drug like properties needs to be performed in order to promote them as a potential drug candidate for marketed products. Further clinical trials of bioactive compounds alone and in synergistic combination are the major field which needs exploration. Also more research and clinical investigation are needed to find out the actual mechanism and determine the optimal range of dosage.

Declaration of competing interest

The author declares no conflict of interest associated with this publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112876.

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