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Review article

Withania somnifera: An Indian ginseng

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Abstract

Withania somnifera, popularly known as Ashwagandha is widely considered as the Indian ginseng. In Ayurveda, it is classified as a rasayana (rejuvenation) and expected to promote physical and mental health, rejuvenate the body in debilitated conditions and increase longevity. Having wide range of activity, it is used to treat almost all disorders that affect the human health. The present review discusses the pharmacological basis of the use of *W. somnifera* in various central nervous system (CNS) disorders, particularly its indication in epilepsy, stress and neurodegenerative diseases such as Parkinson's and Alzheimer's disorders, tardive dyskinesia, cerebral ischemia, and even in the management of drug addiction.
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Keywords: Adaptogen; Antioxidant; Epilepsy; GABA; Neurodegenerative disorders; *Withania somnifera*

Contents

Abbreviations: CNS, central nervous systems; LD50, Lethal dose in producing mortality in 50% of animals; GABA, γ-aminobutyric acid; WS, *Withania somnifera.*

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1. Introduction

Withania somnifera (WS) is popularly known as Ashwagandha or Winter Cherry (Andallu and Radhika, 2000). It is a green shrub (family Solanaceae) (Dafni and Yaniv, 1994) found throughout the drier parts of India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa, Egypt, Morocco and Jordan. In India, it is widely grown in the provinces of Madhya Pradesh, Uttar Pradesh, plains of Punjab and northwestern parts of India like Gujarat and Rajasthan (Bhatia et al., 1987). The plant is popularly known in India by different vernacular names like Punir (Hindi), Ashvaganda (Bengal, Bombay), Aksan (Punjab), Amukkira (Tamil), Tilli (Marathi) [http://www.ayurvediccure.com/ashwagandha_herb.htm] etc. The practitioners of the traditional system of medicine in India regard *W. somnifera* as the "Indian Ginseng" (Singh et al., 2001). Various parts of the plant have been used for centuries to treat variety of ailments (Bhattacharya et al., 2001; Kulkarni et al., 1998; Bhattacharya et al., 1987).

Many pharmacological studies have been carried out to describe multiple biological properties of *W. somnifera* (Mishra et al., 2000). These studies have shown that the plant preparation has antiinflammatory (Bhattacharya et al., 1997a), anticancer (Devi et al., 1992; Mohan et al., 2004), antistress and immunomodulatory (Dhuley, 1998; Rai et al., 2003; Archana and Namasivayam, 1999; Ziauddin et al., 1996), adaptogenic (Bhattacharya and Muruganandam, 2003), central nervous system (Bhattacharya et al., 1987; Naidu et al., 2003; Ahmad et al., 2005; Dhuley, 2001; Chaudhary et al., 2003a; Jain et al., 2001), endocrine (Panda and Kar, 1998) and cardiovascular (Mishra et al., 2000; Mohanty et al., 2004) activities, respectively. *W. somnifera* is known to modulate the oxidative stress markers of the body. The root extract significantly reduced the lipid peroxidation (Dhuley, 1998) and increased the superoxide dismutase (SOD) and catalase activity, thus possessing a free radical scavenging property (Panda and Kar, 1997). The active constituents of plant (Withaferin A, Sitoindosides VII–X) are reported to have an antioxidant activity which may contribute at least in part to the reported antistress, immunomodulatory, cognition facilitating, antiinflammatory and antiageing properties (Bhattacharya et al., 1997b). Besides, *W. somnifera* preparations are reported to modulate the GABAergic [(γ-amino-butyric acid (GABA)] (Mehta et al., 1991; Kulkarni and George, 1996) or cholinergic (Schliebs et al., 1997) neurotransmission, accounting for various CNS related disorders (Tohda et al., 2005). The present article is an attempt to critically review the pharmacological basis of the use of *W. somnifera* as an adaptogen, antistress, antiepileptic and protective in neurodegenerative and neuropsychiatric disorders. The review also updates the information on the chemical constituents, biological properties and toxicity profiles of the plant preparations.

2. Constituents

The major biochemical constituents of *W. somnifera* are steroidal alkaloids and lactones, a class of constituents together known as withanolides (steroidal lactones with ergostane skeleton) (Elsakka et al., 1990). The withanolides have the structural resemblance with the active constituents present in the plant *Panax ginseng* known as ginsenosides (Grandhi et al., 1994). The withanolides have C28 steroidal nucleus with C9 side chain, having six membered lactone ring (Thakur et al., 1987; Puri, 2002). Therefore, because of this *W. somnifera* is named as an "Indian Ginseng" (Grandhi et al., 1994; Singh et al., 2001). So far 12 alkaloids, 35 withanoloids and several sitoindosides have been isolated and their structures have been elucidated (Mishra et al., 2000; Matsuda et al., 2001). The various alkaloids include withanine, somniferine, somnine, somniferinine, withananine, psuedo-withanine, tropine, psuedotropine, 3-α-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine and anahydrine. Two acyl steryl glucoside *viz*. sitoindoside VII and sitoindoside VIII, two glycowithanoloids *viz*. sitoindoside IX or sitoindoside X have been isolated from the root.

Withaferin A has been recently reported to be inhibitor of angiogenesis and thus protective in certain types of cancers (Mohan et al., 2004). Two glycowithanoloids (sitoindoside IX or sitoindoside X) possessed antistress activity and augmented learning acquisition and memory retention in both young and old rats (Ghosal et al., 1989). Recently, two new steroidal lactones of the withanolide-type have been isolated from the fruits of *W. somnifera* (Abou-Douh, 2002). The diverse active constituents present in different parts of the plant are believed to be responsible for the multiple medicinal properties of *W. somnifera*.

The structures of important constituents of *W. somnifera* root extract are given in Fig. 1.

3. Toxicity profile of *W. somnifera*

W. somnifera is considered to be a safe drug. In one of the studies, a 2% suspension of ashwagandholine (total alkaloids from the roots of *W. somnifera*) prepared in ten-percent glycol using two percent gum acacia as suspending agent was used to determine acute toxicity. The acute LD50 value was found to be 465 mg/kg (332–651 mg/kg) in rats and 432 mg/kg (229–626 mg/kg) in mice (Malhotra et al., 1965). The extract had no profound effect on central nervous system or autonomic nervous system in doses of up to 250 mg/100 g of mice in toxicity studies. However, it affected spontaneous motor activity in still higher doses. In another long-term study, *W. somnifera* was boiled in water and administered to rats in their daily drinking water for eight months while monitoring body weight, general toxicity, well being, number of pregnancies, litter size, and progeny weight (Sharma et al., 1986). The estimated dose received by the animal was 100 mg/kg/day. The liver, spleen, lungs, kidneys, thymus, adrenals, and stomach were examined histopathologically and were all found to be normal. The rats treated with *W. somnifera* showed weight gain as compared to the control group. The offsprings of the group receiving *W. somnifera* were found to be healthier compared to control group (Sharma et al., 1986). The different doses of the extract (30, 75 and 150 mg/kg) potentiated pentobarbitone-induced sleeping time in a dose-dependent fashion (Prabhu

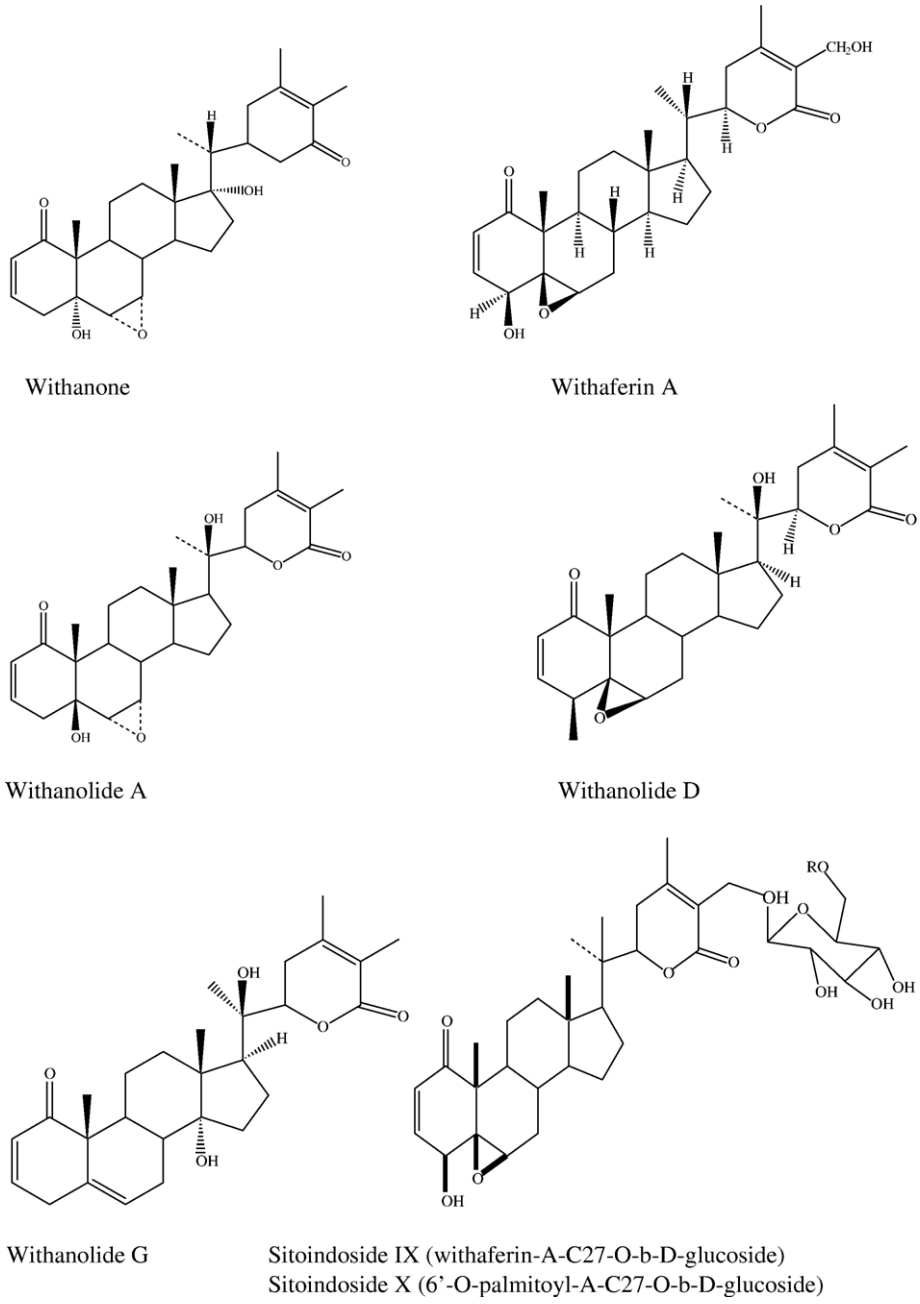

Fig. 1. Active constituents of *Withania somnifera* (Mishra et al., 2000).

et al., 1990). Aphale et al. (1998) carried out the subacute toxicity studies with *W. somnifera* and ginseng. Both the drugs were administered for a period of 90 days and various safety parameters such as food consumption, body weight, hematological, biochemical and histopathological studies on various organs like brain, heart, lung, liver, spleen, kidney, stomach, testis and ovaries were recorded. The results revealed that both the plant preparations did not show any toxicity pattern (Aphale et al., 1998). But, in one study, when the entire plant extract was administered to mice as 25% of the diet, microscopic lesions were found in the liver and lungs along with vascular and tubular congestions of the kidneys. The leaf extract of *W. somnifera* was reported to possess antigenotoxic potential (Rani et al., 2005; Russo et al., 2001). These extensive toxicological studies demonstrated that the plant is nontoxic in wide range of reasonable doses and it can be assumed that the doses in which its preparations are indicated in humans are expected to be very safe. As of today no herb-herb or herb–drug interactions have been reported in the literature with *W. somnifera* (Arseculeratne et al., 1985).

4. *W. somnifera* and central nervous system (CNS) related disorders

W. somnifera preparations have been found to have potential therapeutic role in almost every CNS related disorders.

W. somnifera modulated GABAergic, cholinergic and oxidative systems. The phytochemicals present in *W. somnifera* are responsible for overcoming the excitotoxicity and oxidative damage (Parihar and Hemnani, 2003; Russo et al., 2001). The plant extract inhibited the hydrogen peroxide-induced cytotoxicity and DNA damage in human nonimmortalized fibroblasts (Russo et al., 2001). The active principles of *W. somnifera*, sitoindosides VII–X and withaferin A (glycowithanolides), have been extensively tested for antioxidant activity against the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels of frontal cortex and striatum of the rat brain. Active glycowithanolides of *W. somnifera* (10 or 20 mg/kg., i.p.) when administered once daily for 21 days, an increase in all enzymes was observed, the effect was comparable to those of deprenyl, a known antioxidant (Bhattacharya et al., 1997b). Recently, withanolides have been found to have calcium antagonistic properties (Choudhary et al., 2005). The withanolides inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in a concentration-dependent fashion with IC_{50} values ranging between 29.0 and 85.2 mM for AChE and BChE, respectively. It has been proposed that the cholinesterase inhibitory potential along with calcium antagonistic ability could make the withanolides as possible drug candidates for further study to treat Alzheimer's disease and associated problems (Choudhary et al., 2005). Recent studies have also shown the antiparkinson's like activity of *W. somnifera*, thus possibly modulate dopaminergic system in the brain (Ahmad et al., 2005).

It is known that immobilization stress for 14 h causes 85% degeneration of the cells (dark cells and pyknotic cells) in the CA(2) and CA(3) subareas of hippocampal region as compared to control rats. Control rats were maintained in completely, nonstressed conditions. Pretreatment with root extract of *W. somnifera* (Stresscom® capsules, Dabur India Ltd.) significantly reduced (80%) the number of degenerating cells in both the areas, demonstrating thereby the neuroprotective effects of plant preparation (Jain et al., 2001). EuMil®, a polyherbal medicine consisting of standardized extract of *W. somnifera*, *Oscimum sanctum*, *Asparagus racemosus* and *Emblica officinalis* is widely prescribed as antistress formulation in the Indian system of medicine (Bhattacharya et al., 2002).

5. Neurodegenerative diseases

The protective effect of *W. somnifera* has been summarized in Fig. 2. A multicomponent herbal preparation, BR-16A (Mentat®), which contained *W. somnifera* as one of the main components exhibited protective effective against haloperidol- or reserpine-induced catalepsy in mice. BR-16A (50 and 100 mg/kg. p.o.) and *W. somnifera* (50 and 100 mg/kg p.o.) significantly and dose dependently reduced haloperidol or reserpine-induced catalepsy (Kumar and Kulkarni, 2006). In another study, the antiparkinson effects of *W. somnifera* extract were evaluated using 6-hydroxy dopamine (6-OHDA)-induced Parkinson's-like effect in rats (Ahmad et al., 2005). *W. somnifera* (100, 200 and 300 mg/kg) extract reversed all the parameters of oxidative stress (lipid peroxidation, reduced

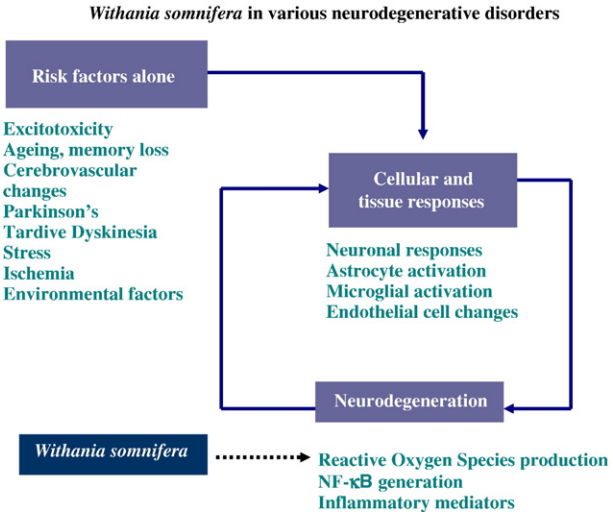

Fig. 2. Possible effect of *Withania somnifera* in various neurodegenerative disorders.

glutathione content, activities of glutathione-*S*-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase) and dopaminergic D_2 dopamine receptor binding and tyrosine hydroxylase expression significantly in a dose-dependent manner as compared to 6-OHDA treated rats (Ahmad et al., 2005). Similarly, *W. somnifera* root extract (100 mg/kg body weight) was also found to be protective in 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-induced catalepsy (Sankar et al., 2007). Recently, *W. somnifera* has been reported to be effective (as a concoction containing cow's milk, powdered *Mucuna pruriens* seeds and *W. somnifera*) in patients clinically diagnosed as Parkinson's patients (Nagashayana et al., 2000).

Tardive dyskinesia is a syndrome characterized by repetitive involuntary movements, usually involving the mouth, face and tongue and sometimes limb and trunk musculature. The dopaminergic receptors supersensitivity and the oxidative stress are considered to be the pathophysiological reasons in this motor dysfunction. Despite the alertness that neuroleptics could cause extrapyramidal side effects (EPS), these drugs remain the most effective treatment in schizophrenia and for the management of behavioral disorders in developmentally disabled individuals (Kulkarni and Naidu, 2001). The studies carried out in our laboratory have demonstrated that chronic administration of the root extract of *W. somnifera* for 4 weeks in reserpine treated animals reduced the onset of vacuous chewing movements (VCMs) (Fig. 3A) and tongue protrusion (Fig. 3B), which are the main behavioral symptoms of Tardive dyskinesia. Chronic *W. somnifera* root extract also reversed reserpine-induced retention deficit. When checked against various biochemical parameters, the root extract dose dependently (50 and 100 mg/kg) reduced the lipid peroxidation and restored the decreased glutathione (GSH) levels. It also significantly reversed the reserpine-induced decrease in brain SOD and catalase levels in rats (Naidu et al., 2006). Similar protective effects were observed in haloperidol-induced vacuous chewing

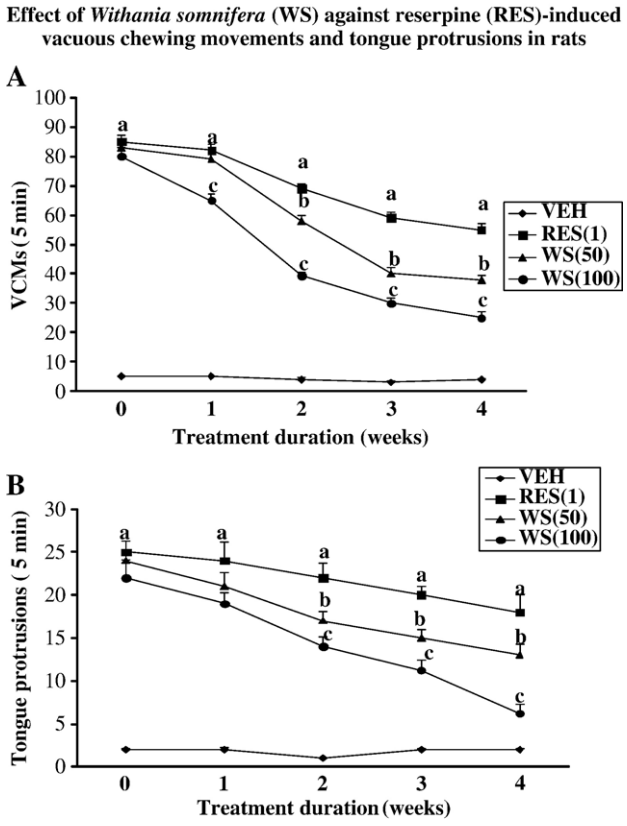

Fig. 3. Effect of chronic administration of *Withania somnifera* (WS) against reserpine (RES)-induced (A) vacuous chewing movements and (B) tongue protrusions in rats. Values expressed as mean±SEM. [a]$p<0.05$ compared with vehicle treated control group. [b]$p<0.05$ compared with reserpine and WS 100 mg/kg treated groups. [c]$p<0.05$ compared with reserpine, WS 50 mg/kg (ANOVA followed by Tukey's test) as compared to reserpine alone treated rats (Naidu et al., 2006).

movements and tongue protrusions in the rats. These observations were supported by the work of Bhattacharya et al., 2002.

6. Neuropsychiatric diseases

6.1. Epilepsy

W. somnifera has profound CNS depressant actions. It has been shown to possess anticonvulsant properties in acute and chronic models of epilepsy (Kulkarni and Verma, 1993; Kulkarni et al., 1993). The root extract has antiepileptic activity against pentylenetetrazol (PTZ)-induced kindling in mice (Kulkarni and George, 1996), amygdaloid kindling in rats (Kulkarni and George, 1995), and in status epilepticus in rats (Kulkarni et al., 1998). The protective effect of *W. somnifera* in epilepsy is considered to be through GABAergic modulation.

6.1.1. Pentylenetetrazol-induced convulsions

W. somnifera possessed protective activity against pentylenetetrazol (PTZ) (80 mg/kg i.p.)-induced convulsions in laka strain of mice. PTZ when injected intraperitoneally produced severe tonic–clonic convulsions in mice. PTZ effects are thought to be mediated through specific interaction with $GABA_A$ gated chloride ionophore. PTZ-induced convulsions represent the petit-mal type of seizures and have been primarily utilized as animal model to evaluate antiepileptic drugs. However, it has been shown that most anxiolytic drugs are also able to prevent or antagonized PTZ-induced convulsions (Vogel et al., 2002). The ethanolic extract of *W. somnifera* (Aswal, provided by Gufic Ltd., Bombay) was suspended in distilled water and administered orally 1 h prior to the administration of PTZ. In another group of animals, GABA and pentobarbitone were administered intraperitoneally, half an hour prior to the administration of pentylenetetrazol. The data was analyzed by Student's *t*-test. *W. somnifera* (30, 100 and 200 mg/kg) dose dependently offered significant ($p<0.05$ as compared to control value) protection against convulsions as shown by an increase in the onset time of extensor and protection against mortality due to PTZ. Pretreatment with *W. somnifera* extract at 200 mg/kg completely abolished the extensor phase of PTZ-induced convulsions (Table 1) (Kulkarni et al., 1993). There was a significant delay in the onset of jerks ($p<0.05$ as compared to control value), and reduction of mortality rate to 57.2%. Concomitant administration of GABA (100 mg/kg) with the subprotective dose of the extract (30 mg/kg) produced a significant delay in the onset of the extensor phase and a significant reduction of the mortality to 80%. Concomitant administration of a higher dose of GABA (200 mg/kg) with the extract (30 mg/kg) produced a significant delay in the onset of clonus and a significant reduction of mortality to 50%. On increasing the dose of the extract to 100 mg/kg there was no significant delay in the onset of all the three stages or the mortality rate (Kulkarni et al., 1993) (Table 2).

However, when *W. somnifera* was combined with different doses of pentobarbitone it enhanced the protective effect of pentobarbitone (5 and 10 mg/kg) ($p<0.001$ as compared to pentobarbitone alone group). The combination of *W. somnifera*

Table 1
Effect of *Withania somnifera* (30–200 mg/kg) extract against PTZ-induced convulsions (Kulkarni et al., 1993)

Treatment (mg/kg)	No. of animals	Onset of convulsions (s±SEM)			Mortality D/N	% Mortality
		Jerks	Clonus	Extensor		
PTZ (80)	36	46.0±1.81	73.7±5.80	253.0±18.70	36/36	100%
Extract (30)+PTZ (80)	6	56.0±3.62	97.0±18.70	389.8±46.73**	6/6	100%
Extract (100)+PTZ (80)	14	50.5±2.38	84.0±10.72	361.6±23.75*	10/14	71.4%
Extract (200)+PTZ (80)	6	50.5±2.25	89.8±6.63	–	–	–

*$p<0.05$, **$p<0.01$ as compared to control value (PTZ) (ANOVA followed by Dunnett's test).

Table 2
Effect of GABA (100 or 200 mg/kg) and its combination with *Withania somnifera* (30 or 100 mg/kg) extract against PTZ-induced convulsions (Kulkarni et al., 1993)

Treatment (mg/kg)	No. of animals	Onset of convulsions (s±SEM)			Mortality D/N	% Mortality
		Jerks	Clonus	Extensor		
PTZ (80)	36	46.0±1.81	73.7±5.80	253.0±18.70	36/36	100%
GABA (100)+PTZ (80)	8	58.5±5.77*	104.3±18.15	312.9±25.46	7/8	87.5%
GABA (200)+PTZ (80)	7	63.0±6.20*	82.4±6.60	680.3±29.50***	4/7	57.2%
Extract (100)+GABA (100)+PTZ (80)	7	63.9±6.62	105.1±16.04	277.5±24.77	4/7	57.2%
Extract (100)+GABA (200)+PTZ (80)	10	61.1±4.05*	95.4±15.44	369.8±40.20	4/10	40%
Extract (30)+GABA (100)+PTZ (80)	5	63.4±3.33	89.0±8.13	415.0±20.20*	4/5	80%
Extract (30)+GABA (200)+PTZ (80)	8	61.9±1.47	109.8±3.67*	444±21.97	4/8	50%

*$p<0.05$, ***$p<0.001$ as compared to control or GABA treatment (ANOVA followed by Dunnett's test).

(100 mg/kg) with pentobarbitone (10 mg/kg) completely abolished the extensor phase against PTZ-induced convulsions (Kulkarni et al., 1993) (Table 3) thereby suggesting that extract may act at the barbiturate modulatory centre on the $GABA_A$ receptor.

6.1.2. Kindling studies

Kindling is a chronic model of epilepsy and epileptogenesis. In PTZ or electrical kindling, the repeated application of convulsants (PTZ or electrical stimulation) applied once a day or alternate days, which initially has no effect on behavior but slowly culminating into full blown convulsions. PTZ-induced kindling is one such type of model in which subconvulsant dose of PTZ (40 mg/kg) on alternate days leads to full blown convulsions and the animals go into a continuous state of convulsions (kindling) and become seizure prone. PTZ-induced kindling is an experimental model of epilepsy that shares many features in common with electrical limbic kindling but that primarily involves neocortex (Barkai et al., 1994). Although the pathogenesis of kindled seizures is not fully understood, it serves as a useful tool for investigating the efficacy of experimental anticonvulsant agents.

The study conducted in our laboratory, pentylenetetrazol was administered thrice a week for 9 weeks and after each injection of PTZ, animals were observed for 10–15 min for any CNS excitation. The intensity of behavioral seizures was evaluated using a six point scoring system, (0, no effect; 1, jerks; 2, Straub's tail; 3, clonus). Cumulative kindling score (calculated by taking the average of all the individual behavioral scores and then dividing them with the number of subjects) was calculated. These chronically treated mice were challenged with the same subconvulsive dose of PTZ (30 mg/kg, i.p.) on days 3 and 10 of the last chronic dose. These mice showed different phases of CNS excitation and convulsions which were recorded.

W. somnifera protected these animals against PTZ-induced kindling, although, the effect was less potent as compared to diazepam treatment (Table 4A, B) (Kulkarni and George, 1996).

6.1.3. Amygdala-pyriform kindling

Amygdala-pyriform cortex is the most responsive within the limbic system in the rate of kindling development as compared to hippocampus and cortex. The regularity and predictability of amygdaloid kindling under a given set of stimulus conditions make this model ideal for the study of neuronal mechanisms that may underlie seizure development. In one of the studies reported from our laboratory, an attempt was made to study the protective effect of *W. somnifera* root extract in amygdaloid kindled rats. Bipolar concentric stainless steel electrodes were used for stimulation and also for producing lesions in the amygdale. Electrodes were implanted into the basolateral amygdale, using standard stereotaxic techniques, and the following coordinates: 2.8 mm posterior to bregma, 4.8 mm lateral and 8.5 mm below dura, as per the atlas of Paxinos and Watson. The electrical stimulation (300 μA; 60 Hz, 1 ms pulse duration for 2–3 s, once a day) was given for total of 15 days and animals showed persistent kindling. Electroencephalographic recording showed epileptic changes in both the amplitude and frequency of wave patterns, confirming the long-term periodic development of spontaneous motor seizures. Repeated electrical stimulations of the amygdale in rats are reported to produce changes in other brain areas (Goddard et al., 1969). The changes could be due to a shift in the sensitivity of the secondary structures or it could be a reflection of their strong interconnections. Treatment with *W. somnifera* given acutely (100 mg/kg, p.o.), 30 min prior to stimulus, showed

Table 3
Effect of pentobarbitone (5 and 10 mg/kg) and its combination with *Withania somnifera* (100 mg/kg) extract against PTZ-induced convulsions (Kulkarni et al., 1993)

Treatment (mg/kg)	No. of animals	Onset of convulsions (s±SEM)			Mortality D/N	% Mortality
		Jerks	Clonus	Extensor		
PTZ (80)	36	46.0±1.81	73.7±5.80	253.0±18.70	36/36	100%
Pentobarbitone (5)+PTZ (80)	6	56.3±5.19*	85.3±16.18	535.0±35.18***	4/6	66.7%
Pentobarbitone (10)+PTZ (80)	9	69.4±5.79*	223.6±53.38**	387.5±52.5*	2/9	22.2%
Extract (100)+Pentobarbitone (5)+PTZ (80)	6	76.3±4.53*	97.3±6.80	645.0***±20.0	2/6	33.3%
Extract (100)+Pentobarbitone (10)+PTZ (80)	10	96.1±9.14***	246.0±57.04	–	–	–

*$p<0.05$, **$p<0.01$ ***$p<0.001$ as compared to control or pentobarbitone alone groups (ANOVA followed by Dunnett's test).

Table 4
Effect of *Withania somnifera* (100 mg/kg) on onset time of clonus, jerks, severity of clonus and % animals showing jerks or clonus against PTZ-induced kindling (A) on 3rd day of last challenge day of PTZ (B) on 10th day of last challenge day of PTZ

Treatment (mg/kg)	*N*	Cumulative kindling score	Onset (s±SE)		Severity of clonus	% Showing	
			Jerks	Clonus		Jerks	Clonus
(A)							
Control PTZ i.p.	14	4.92±0.54	173.1±26.97 (14/14)	331.2±31.8 (9/14)	++	100	64.3
WS (100) p.o.	12	0.75±0.58[a]	346.7±50.44 (3/12)	–	–	25	0
Diazepam (1.0) i.p.	9	0.55±0.22[a]	435 (1/9)	–	–	11.1	0
(B)							
Control PTZ i.p.	14	4.73±0.42	194.1±25.84 (14/14)	324.8±59.28 (9/14)	++	100	64.3
WS 100 p.o.	10	1.6±0.31[a]	352.2±67.77 (7/10)	448 (1/10)	+	70	10
Diazepam 1.0 i.p.	8	0.25±0.24[a]	–	–	–	0	0

[a] $p<0.05$ as compared to control value (Kulkarni and George, 1996).

significant reduction in severity of motor seizures as evident from the amplitude and frequency of wave pattern (Kulkarni et al., 1994).

6.1.4. Status epilepticus

In another experimental model, i.e. status epilepticus, *W. somnifera* was given acutely and it delayed the onset of forelimb clonus and rearing but the drug was not able to reduce the mortality rate against lithium–pilocarpine-induced status epilepticus in rats. When the root extract of *W. somnifera* (50, 100, 200 mg/kg. p.o.) was given chronically for 7 days followed by lithium–pilocarpine challenge, it protected the animal from mortality up to 60% but did not reduce the latency of forelimb clonus with rearing. Furthermore, *W. somnifera* was also combined with the standard antiepileptic drugs. All the anticonvulsant drugs namely, diazepam (1, 2.5 and 5 mg/kg), clonazepam (0.25, 0.5 and 1 mg/kg), sodium valproate (100, 300 mg/kg) and phenytoin (50, 100 mg/kg) produced a dose-dependent protection. When *W. somnifera* was combined with these standards agents, the combination was able to reduce significantly the effective dose of diazepam and clonazepam to offer full protection with no mortality (Table 5) (Kulkarni et al., 1998).

When checked for EEG recordings the cortical amplitude showed reduced amplitude (Fig. 4) with *W. somnifera* pretreatment until 60 min compared with the control groups (Kulkarni et al., 1998).

6.1.5. Molecular studies

The profound anticonvulsant activity of *W. somnifera* root extract as reported in various animal models is hypothesized to be through $GABA_A$ receptors (Mehta et al., 1991). The

Table 5
Effect of *Withania somnifera* (WS) alone and in combination with known anticonvulsants against lithium–pilocarpine-induced status epilepticus (SE) in rats

Treatment (mg/kg)	Onset of F.C+R	Mortality (%)
Control (lithium+pilocarpine)	25.4±0.97	100
Acute study		
WS (100)	56±2.12[NS]	100
Diazepam (2.5)	60.4±15.73[NS]	50
WS (100)+diazepam (2.5)	85±5.01[a]	20
Sodium valproate (100)	38.2±5.2[a]	80
Sodium valproate (300)	74.83±9.09[a]	66
WS (100)+sodium valproate (300)	75.8±5.94[a]	66
Clonazepam (0.25)	72±11.09[a]	40
WS (100)+Clonazepam (0.25)	100±0[b]	0
Phenytoin (100)	79±7.16[a]	40
WS (100)+phenytoin (100)	78.2±7.45[a]	40
Chronic study		
WS (50) X 7d	45±9.6[NS]	83
WS (100) X 7d	50.2±5.87[NS]	60
WS (200) X 7d	57.2±11.3[NS]	66.6

F.C+R, forelimb clonus with rearing.
[a]$p<0.05$, [b]$p<0.01$ (Kruskal–Wallis test followed by Student's *t*-test) (Kulkarni et al., 1998), NS–Not Significant.

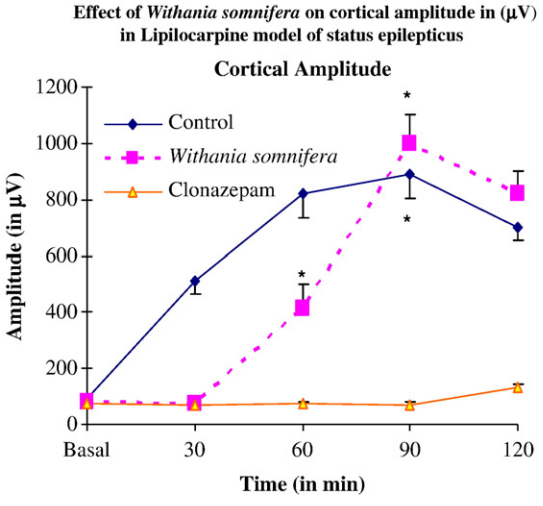

Fig. 4. Time course change in the cortical amplitude in (μV) in Lithium-pilocarpine model of status epilepticus following different drug pretreatment in rats.*$p<0.05$ (significance with respect to basal values, Friedman's test). Overall significant difference exists between the area under the curves of Control (Cnt) and Clonazepam (Cz); Clonazepam (Cz) and *Withania somnifera* (WS) (Kulkarni et al., 1998).

$GABA_A$-benzodiazepine receptor-ionophore complex is a major site of drug action for a variety of centrally acting drugs including benzodiazepines and barbiturates (Olsen, 1981; Ticku, 1983). Barbiturates and benzodiazepines facilitate the effect of GABA mediated Cl^- conductance. *W. somnifera* root extract is reported to have anxiolytic (Bhattacharya et al., 1987) and CNS inhibitory effects (Malhotra et al., 1965). To test this hypothesis Mehta et al. (1991) conducted experiments to determine the modulatory effect of a methanol extract of *W. somnifera* on the binding of [^{3}H] GABA, [^{35}S] *t*-butylbicyclo-phosphorothionate (TBPS) and [^{3}H] flunitrazepam to their respective sites in brain neurons. The methanol extract of *W. somnifera* produced a concentration-dependent inhibition of both [^{3}H] GABA and [^{35}S] TBPS binding in rat cerebral cortical membranes. The extract was more potent in inhibiting [^{3}H] GABA and [^{35}S] TBPS binding. The methanol extract of *W. somnifera* increased the specific binding of [^{3}H] flunitrazepam in cerebral cortex. Also the effect of extract on [^{3}H] flunitrazepam binding was additive with that of pentobarbital. When the extract and GABA were tested in combination on [^{3}H] flunitrazepam binding the effect was not additive. The effect of combination treatment indicated that the extract decreased the enhancing effect of GABA. Similar results were obtained when the effect of two GABA agonists (GABA and muscimol) was examined in [^{3}H] flunitrazepam binding. These functional studies indicated that *W. somnifera* possessed its anticonvulsant activities through $GABA_A$ receptor system.

The schematic diagram showing the possible mechanism of action concerning the protective effect of *W. somnifera* in epilepsy is shown in Fig. 5.

6.2. Anxiety

Anxiety disorders have a lifetime prevalence that exceeds 15% in the general population (Kessler et al., 1994). In India, Ashwagandha (*W. somnifera*) has been extensively used for centuries in Ayurvedic medical practice to reduce symptoms of anxiety and stress (Handa, 1995). As demonstrated earlier *W. somnifera* possessed GABA-mimetic properties (Kulkarni et al., 1993; Mehta et al., 1991). Since GABA agonism has been linked to anxiolysis (Stahl, 1998), the extracts of *W. somnifera* may have beneficial effect in anxiety and related disorders. A double blind placebo control study conducted by other investigators, in patients with ICD-10 anxiety disorders, 6 weeks of treatment with ethanolic extract of *W. somnifera*, the patients met with priori response criteria in the drug group (88.2%) as compared with the placebo group (50%). The sample of the study comprised of 39 subjects, of whom 20 received the drug and 19 received the placebo. The two groups were sociodemographically and clinically similar at baseline. At 2 and 6 weeks follow-up, data from approximately 85% of patients in each group were available for analysis. Statistical trend favoring the drug was observed at both time points. Essentially, this study demonstrated a trend for the anxiolytic superiority of drug over placebo at week 2, and a statistically significant superiority at week 6. The extract was well tolerated and did not cause more adverse effects than placebo. So, it was concluded that the ethanolic extract of *W. somnifera* has useful anxiolytic potential (Andrade et al., 2000). Also, in another study administration of the preparation (20 and 50 mg/kg) orally once daily for 5 days (results were compared with lorazepam, 0.5 mg/kg, i.p.), it showed *an* anxiolytic effect (Bhattacharya et al., 2000).

Shaligram and colleagues (personal observations) carried out a clinical study with *W. somnifera* tablets on the psychomotor performance. The study was carried out in a double blind cross over manner. The volunteers were required to state their position along the three 100 mm scales for anxious-calm mode, relaxed tense mode and the drowsy alert mode. It was concluded that *W. somnifera* tablets had a beneficial effect in the tense and

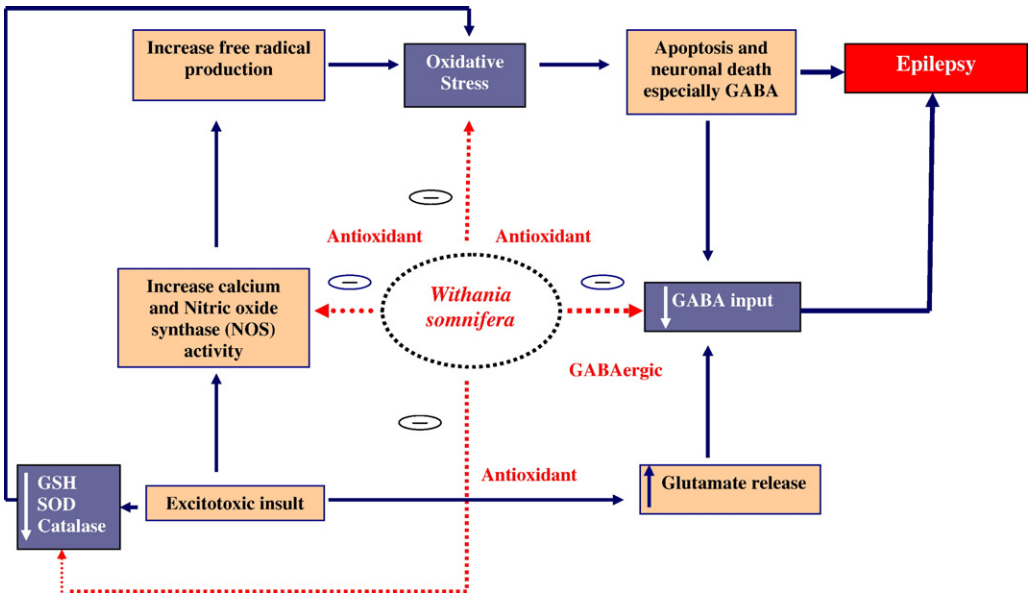

Fig. 5. Schematic diagram showing protective effect of *Withania somnifera* in epilepsy.

anxious state but this was not accompanied by sedation or drowsiness. The effect was compared with diazepam where diazepam reported to produce sedation besides producing the anxiolytic action (unpublished data).

6.3. Antistress and adaptogenic action

W. somnifera is extensively used for relieving stress in the patients, thus acting as an antistress medication. In a study carried out in our laboratory, the effect of *W. somnifera* was observed in a mouse model of chronic fatigue syndrome (CFS). CFS is an illness characterized by persistent and relapsing fatigue. In this study, the mice were made fatigued by forcing them to swim for 6-minute session on each day for a total of 15 days. *W. somnifera* and other antioxidants were administered daily before subjecting the animals to stress. Mean immobility period was calculated on every day and compared with control animals. *W. somnifera* produced a significant decrease in immobility time as compared to control stressed animals, showing the antistress activity of the extract (Singh et al., 2002).

The antistress activity has been explained by the fact that *W. somnifera* root extract has antioxidant property. Chronic fatigue induced by forced swimming for 15 days induced a significant raise in brain MDA levels as compared to naïve mice indicating the oxidation of proteins, DNA and lipids. Administration of *W. somnifera* (100 mg/kg p.o.) significantly reversed the extent of lipid peroxidation (Table 6). Dhuley (2000) has reported the adaptogenic potential of *W. somnifera* in rats and frogs (Dhuley, 2000). Similar results were reported by Archana and Namasivayam, 1999 showing antistress property of *W. somnifera* in cold water swim stress. *W. somnifera* is thus categorized under antistress and adaptogenic agent (Rege et al., 1999).

The antioxidant activity of *W. somnifera* glycowithanolides was also assessed in chronic footshock-induced stress. The stress procedure, given once daily for 21 days, induced an increase in activity of superoxide dismutase (SOD) and lipid peroxidation (LPO), with concomitant decrease in activities of catalase and glutathione peroxidase in both the brain regions. Administration of *W. somnifera* extract in the doses of 10, 20 and 50 mg/kg orally 1 h prior to the stress procedure for 21 days produced a dose-related reversal of the stress effects (Bhattacharya et al., 2001). In another study, the effect of *W. somnifera* root extract was seen in stress-induced neuronal degeneration in rats. The ultrastructural study of neuronal cell bodies in hippocampal sublayer (CA1–CA4) was studied. Study suggested the cytoprotective effect of *W. somnifera* in improving degenerating characteristics (which included karyorrhexis, membrane blebbing, chromatin condensation, chromatin fragmentation and intracellular spacing) in rat brain (Shukla et al., 2000). Two glycosides, sitoindoside VII and sitoindoside VIII isolated from the roots of *W. somnifera* showed significant antistress activity when tests in diverse spectrum of stress-induced paradigms (Bhattacharya et al., 1987). Similarly, sitoindoside IX and sitoindoside X in a dose range of 50–200 mg/kg p.o. produced significant antistress activity in mice and rats and augmented learning acquisition and memory retention in both young and old rats (Ghosal et al., 1989). Thus, *W. somnifera* is supplemented in different therapeutic formulations for treating stress and related disorders (Muruganandam et al., 2002).

Table 6
Effect of *Withania somnifera* (WS) root extract on chronic swimming-induced malondialdehyde (MDA) levels in mouse brain

Treatment (mg/kg)	nmol MDA/mg protein
Naïve mice (without stress)	1.459±0.081
Chronic stressed mice	4.356±1.112 [a]
WS (100)+chronic stress	1.558±0.022 [b]

[a] $p<0.05$ compared with naïve mice.
[b] $p<0.05$ compared with stressed mice (Singh et al., 2002).

6.4. Memory

Withanoloids isolated from the *W. somnifera* are known to inhibit acetylcholinesterase and butylcholinesterase in a dose-dependent manner. The cholinesterase inhibitory potential along with calcium antagonistic ability has made *W. somnifera* possible drug candidate to treat Alzheimer's disease and associated problems (Choudhary et al., 2005). Sitoindosides VII–X, and withaferin-A, isolated from aqueous methanol extract from the roots of cultivated varieties of *W. somnifera* is used in Indian medicine to attenuate cerebral functional deficits, including amnesia, in geriatric patients (Schliebs et al., 1997). The effect of these active constituents of *W. somnifera* was also investigated for putative nootropic activity in a experimentally validated Alzheimer's disease model. The syndrome was induced by ibotenic acid lesioning of the nucleus magnocellularis in rats. *W. somnifera* significantly reversed both ibotenic acid induced cognitive deficit and the reduction in cholinergic markers after 2 weeks of treatment. These findings validated the Medharasayan (promoters of learning and memory) effect of *W. somnifera* (Glotter, 1991). Memory-deficient mice showed neuronal atrophy and synaptic loss in the brain and the treatment with withanolide A induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and postsynapses in the neurons, thus may improve memory process (Kuboyama et al., 2005). It is found that withanoside IV (a constituent of the roots of *W. somnifera*) induced neurite outgrowth in cultured rat cortical neurons. Orally administrated withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease due to sominone, an aglycone of withanoside IV (Kuboyama et al., 2006). Similarly, withanolide-A (1 microM) induces significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and postsynapses in the neurons and therefore, an important candidate for the therapeutic treatment of neurodegenerative diseases (Kuboyama et al., 2005). An *in vitro* study from Toyama Medical and Pharmaceutical University in Japan showed administration of methanolic extract of *W. somnifera* root (5 mcg/mL) significantly and dose-dependently increased the percentage of cells with neurites in human neuroblastoma SK-N-SH cells; mRNA levels of dendritic markers were

Table 7
Effect of *Withania somnifera* (WS; 100 mg/kg) root extract on number of jumps induced by injecting naloxone in morphine addicted mice (Kulkarni and Ninan, 1997)

Group No.	Treatment (mg/kg)	*n*	Number of jumps (mean±SEM)
1	Saline: saline	6	0
2	Saline: morphine (10 mg/kg)	6	8.3±2.8
3	WS (100): saline	6	0
4	WS (100): morphine (10)	6	0

markedly increased by treatment with the extract, whereas those of the axonal marker *Tau* were not (Tohda et al., 2000). Additional studies from the same institution found oral administration of withanoside extracted from *W. somnifera* significantly improved memory deficits in β amyloid-injected mice (to induce dendritic and axonal atrophy) and prevented loss of axons, dendrites, and synapses in the cerebral cortex and hippocampus (Kuboyama et al., 2005, 2006). In another behavioral experiment, *W. somnifera* root extract (50, 100 and 200 mg/kg., orally) improved retention of a passive avoidance task in a step-down paradigm in mice. *W. somnifera* (50, 100 and 200 mg/kg., p.o.) reversed the scopolamine (0.3 mg/kg)-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS), immediately after training, thus showing memory enhancing property (Dhuley, 2001). It was suggested that extract from *W. somnifera* affect preferentially events in the cortical and basal forebrain cholinergic signal transduction cascade. *W. somnifera* may induce an increase in cortical muscarinic acetylcholine receptor capacity that might partly explain the cognition-enhancing and memory-improving effects observed in animals and humans (Schliebs et al., 1997). *W. somnifera* also reversed the memory loss that is induced by oxidative damage caused by streptozotocin (Parihar et al., 2004). Memory impairments in the diabetics is associated with an increased free radical mediated oxidative damage and the supplementation of *W. somnifera* showed protective effects in attenuating diabetes-induced memory loss possibly via its antioxidative mechanisms (Parihar et al., 2004).

6.5. Cerebral ischemia

Stroke causes brain injury in millions of people worldwide each year. Despite the enormity of the problem, there is currently no approved therapy that can reduce infarct size or neurological disability. The medical treatment of stroke includes thrombolysis with tissue plasminogen activator (t-PA). However, t-PA treatment is limited by a narrow time window and side effects. Free radicals have been implicated in neuronal injury during ischemia reperfusion in stroke (Sinha et al., 2002) and various antioxidants such as trans resvaratrol (Sinha et al., 2002), alpha-tocopherol (Chaudhary et al., 2003b), adenosine (Gupta et al., 2002), melatonin (Sinha et al., 2001) are being tested in different animal models of stroke. In one study, *W. somnifera* was evaluated in a middle cerebral artery (MCA) occlusion model of stroke in rats and the results proved to be quite interesting. Two groups of male Wistar rats were pre-treated with a hydroalcoholic extract of *W. somnifera* (1 g/kg, p.o.) for 15 and 30 days. Thereafter, rats were subjected to focal ischemia by occlusion of the middle cerebral artery using an intraluminal thread. After 2 h MCA occlusion, reperfusion was allowed by retracting the thread. Animals were assessed for ischemic changes using diffusion-weighted imaging 30 min after reperfusion. Twenty-four hours later, rats were subjected to motor performance tests and were subsequently killed for the estimation of the marker of oxidative stress malondialdehyde. The control group received vehicle and a similar protocol was followed. Significant motor impairment, with elevated levels of MDA, was observed in vehicle-treated MCA-occluded rats. In the *W. somnifera* (1 g/kg, p.o. for 30 days)-pretreated group, the percentage hemispheric lesion area was significantly attenuated (17±2%) as compared with the vehicle-treated MCA-occluded group (30±4%). The study provided an evidence of the effectiveness of this Indian herb in focal ischemia (Chaudhary et al., 2003a).

6.6. Drug addiction

Chronic administration of opioids is known to produce tolerance and dependence in man and animals. The studies

Table 8
Withania somnifera products and their claimed central nervous system therapeutic effects

Product name	Manufacturer	Stated central nervous system therapeutic claim
Stresswin	Baidynath Ayurved Bhawan	Combating exertion, reduction in anxiety, strain and stress, improvement of stamina, relief from disturbed sleep, mental alertness
Stresscom	Dabur India Ltd	Relieves anxiety neurosis, physical and mental stress, and relieves general debility and depression
Brento	Zandu Pharmaceutical Works Ltd	Nerve tonic
Ashvagandha	Morpheme Remedies	Combating stress
Dabur Ashwagandha Churna	Dabur	Combating stress
Ashwagandha	Ayurceutics	Stress reliever
Himalaya Massage oil	The Himalaya Drug Co	Stress relief and relief from insomnia
Ashwagandharista	Baidynath Ayurved Bhawan	Nerve tonic, memory and cognition improvement, better power of concentration, relieves mental tension, natural sleep induction, and recovery from nervous and general debility
Arshadi Pills	Dehlvi Remedies	Stress, depression, cardiac tonic

Table 9
Therapeutic use and proposed mechanism of action of *Withania somnifera* in various CNS related disorders

Therapeutic use of *Withania somnifera*	Proposed mechanism of action
Alzheimer's disease	▪ Antioxidative mechanism ▪ Inhibiting acetylcholinesterase activity ▪ Inhibiting butyrylcholinesterase activity ▪ Neurites regeneration ▪ Increases cortical muscarinic acetylcholine receptor capacity
Parkinson's disease	▪ Antioxidative mechanism
Tardive dyskinesia	▪ Antioxidative mechanism ▪ Increasing levels of GABA in brain
Epilepsy	▪ Antioxidative mechanism ▪ Increasing GABA levels in the brain ▪ Inhibiting butyrylcholinesterase activity ▪ Neurites regeneration ▪ Increases cortical muscarinic acetylcholine receptor capacity
Stress and related pathologies	▪ Increasing GABA levels in the brain ▪ Antioxidative mechanism
Cerebral ischemia	▪ Antioxidative mechanism
Anxiety	▪ Increasing GABA levels in the brain
Drug addiction	▪ Antioxidative mechanism

conducted in our laboratory have shown that the animals were made addicted to morphine by injecting them with morphine twice daily (09:00 and 16:00 h) for 9 days. Various treatment groups received different treatment schedules such as (pretreatment: treatment) included (i) saline: saline (ii) saline: morphine (10 mg/kg) (iii) *W. somnifera* (100 mg/kg): saline and (iv) *W. somnifera* (100 mg/kg): morphine (10 mg/kg). On 10th day the treatment was reversed so that the animals that had received *W. somnifera* followed by morphine on day 1–9 were challenged with saline followed by morphine and the animals that had treated with saline followed by morphine on days 1–9 were challenged with *W. somnifera* followed by morphine. In addition, the animals that had received chronic treatment with *W. somnifera* followed by saline for 9 days were challenged with *W. somnifera* followed by morphine on day 10. To assess the morphine withdrawal, mice were injected with naloxone (2 mg/kg i.p.) on day 10. The escape jumps were measured for 15 min. The root extract of *W. somnifera* inhibited the development of tolerance as well as dependence to morphine as withdrawal jumps were completely inhibited (Table 7). Chronic treatment with *W. somnifera* root extract inhibited development of tolerance to analgesic effect of morphine. It also inhibited the development of withdrawal jumps (Kulkarni and Ninan, 1997). *W. somnifera* is a safe nonanalgesic herbal preparation which can be used in treatment of opiate addiction.

7. Commercial preparations

The various commercial products of *W. somnifera* and their claimed central nervous system therapeutic effects have been summarized in Table 8.

8. Conclusion

From the above description (concluded in Table 9), it may be concluded that *W. somnifera* could be a useful neuroprotective therapy in various central nervous system related disorders. The drug is without having any serious toxicity or side effects known till date and thus can be safely used in humans for acute and chronic treatment regime.

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References

Abou-Douh AM. New withanolides and other constituents from the fruit of *Withania somnifera*. Arch Pharm (Weinheim) 2002;335:267–76.

Ahmad M, Saleem S, Ahmad AS, Ansari MA, Yousuf S, Hoda MN, et al. Neuroprotective effects of *Withania somnifera* on 6-hydroxydopamine induced Parkinsonism in rats. Hum Exp Toxicol 2005;24: 137–47.

Andallu B, Radhika B. Hypoglycemic diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera* Dunal) root. Indian J Exp Biol 2000;38:607–9.

Andrade C, Aswath A, Chaturvedi SK, Srinivasa M, Raguram R. A double-blind placebo-controlled evaluation of anxiolytic efficacy of an ethanolic extract of *Withania somnifera*. Indian J Psychiatry 2000;42:295–301.

Aphale AA, Chhibba AD, Kumbhakarna NR, Mateenuddin M, Dahat SH. Subacute toxicity study of the combination of ginseng (Panax ginseng) and ashwagandha (*Withania somnifera*) in rats a safety assessment. Indian J Physiol Pharmacol 1998;42:299–302.

Archana R, Namasivayam A. Antistressor effect of *Withania somnifera*. J Ethnopharmacol 1999;64:91–3.

Arseculeratne SN, Gunatilaka AA, Panabokke RG. Studies of medicinal plants of Sri Lanka Part 14 Toxicity of some traditional medicinal herbs. J Ethnopharmacol 1985;13:323–35.

Barkai E, Grossman Y, Gutnic MJ. Long term changes in neocortical activity after chemical kindling with systemic pentylenetetrazol: an in vitro study. J Neurophysiol 1994;72:72–83.

Bhatia P, Rattan SIS, Cavallius J, Clark BFC. *Withania somnifera* (Ashwagandha) a so-called rejuvenator inhibits growth and macromolecular synthesis of human cells. Med Sci Res 1987;15:515–6.

Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera* an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 2003;75:547–55.

Bhattacharya SK, Goel RK, Kaur R, Ghoshal S. Anti-stress activity of sitoindosides VII and VIII New acyl steryl glycosides from *W. somnifera*. Phytother Res 1987;1:32–7.

Bhattacharya SK, Satyan KS, Chakrabarti A. Effect of Trasina, an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. Indian J Exp Biol 1997a;35:297–9.

Bhattacharya SK, Satyan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. Indian J Exp Biol 1997b;35: 236–9.

Bhattacharya SK, Bhattacharya A, Chakrabarti A. Adaptogenic activity of Siotone a polyherbal formulation of Ayurvedic rasayanas. Indian J Exp Biol 2000;38:119–28.

Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol 2001;74: 1–6.

Bhattacharya A, Muruganandam AV, Kumar V, Bhattacharya SK. Effect of poly herbal formulation EuMil on neurochemical perturbations induced by chronic stress. Indian J Exp Biol 2002;40:1161–3.

Chaudhary G, Jagannathan NR, Gupta YK. Evaluation of *Withania somnifera* in a middle cerebral artery occlusion model of stroke in rats. Clin Exp Pharmacol Physiol 2003a;30:399–404.

Chaudhary G, Sinha K, Gupta YK. Protective effect of exogenous administration of alpha-tocopherol in middle cerebral artery occlusion model of cerebral ischemia in rats. Fundam Clin Pharmacol 2003b;17:703–7.

Choudhary MI, Nawaz SA, ul-Haq Z, Lodhi MA, Ghayur MN, Jalil S, et al. Withanolides a new class of natural cholinesterase inhibitors with calcium antagonistic properties. Biochem Biophys Res Commun 2005;334: 276–87.

Dafni A, Yaniv Z. Solanaceae as medicinal plants in Israel. J Ethnopharmacol 1994;44:11–8.

Devi PU, Sharada AC, Solomon FE, Kamath MS. In vivo growth inhibitory effect of *Withania somnifera* (Ashwagandha) on a transplantable mouse tumor Sarcoma 180. Indian J Exp Biol 1992;30:169–72.

Dhuley JN. Effect of ashwagandha on lipid peroxidation in stress-induced animals. J Ethnopharmacol 1998;60:173–8.

Dhuley JN. Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. J Ethnopharmacol 2000;70:57–63.

Dhuley JN. Nootropic-like effect of ashwagandha (*Withania somnifera* L) in mice. Phytother Res 2001;15:524–8.

Elsakka M, Grigorescu E, Stanescu U, Stanescu U, Dorneanu V. New data referring to chemistry of *Withania somnifera* species. Rev Med Chir Soc Med Nat Iasi 1990;94:385–7.

Ghosal S, Lal J, Srivastava R, Bhattacharya SK, Upadhyay SN. Immunomodulatory and CNS effects of sitoindosides IX and X two new glycowithanolides from *Withania somnifera*. Phytother Res 1989;3:201–6.

Glotter E. Withanolides and related ergostane-type steroids. Nat Prod Rep 1991;8:415–39.

Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 1969;25:295–330.

Grandhi A, Mujumdar AM, Patwardhan B. A comparative pharmacological investigation of Ashwagandha and Ginseng. J Ethnopharmacol 1994;44: 131–5.

Gupta YK, Sinha K, Chaudhary G, Jagannathan NR. Protective effect of adenosine against neuronal injury induced by middle cerebral artery occlusion in rats as evidenced by diffusion-weighted imaging. Pharmacol Biochem Behav 2002;72:569–74.

Handa SS. Plants and plant products for mental health. In: Koslow SH, Murthy RS, Coelho GV, editors. Decade of the Brain Rockville MD US Department of Health and Human Service; 1995. p. 163–71.

Jain S, Shukla SD, Sharma K, Bhatnagar M. Neuroprotective effects of *Withania somnifera* Dunn in hippocampal sub-regions of female albino rat. Phytother Res 2001;15:544–88.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States Results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51:8–19.

Kuboyama T, Tohda C, Komatsu K. Neuritic regeneration and synaptic reconstruction induced by withanolide A. Br J Pharmacol 2005;144:961–71.

Kuboyama T, Tohda C, Komatsu K. Withanoside IV and its active metabolite sominone attenuate Abeta (25–35)-induced neurodegeneration. Eur J Neurosci 2006;23:1417–26.

Kulkarni SK, George B. Amygdaloid kindling in rats: protective effect of *Withania somnifera* (Ashwagandha) root extract. Indian Drugs 1995;32: 37–49.

Kulkarni SK, George B. Anticonvulsant action of *Withania somnifera* (Ashwagandha) root extract against pentylenetetrazol-induced kindling in mice. Phytother Res 1996;10:447–9.

Kulkarni SK, Naidu PS. Tardive dyskinesia: an update. Drugs Today (Barc) 2001;37:97-119.

Kulkarni SK, Ninan I. Inhibition of morphine tolerance and dependence by *Withania somnifera* in mice. J Ethnopharmacol 1997;57:213–7.

Kulkarni SK, Verma AV. GABA receptor mediated anticonvulsant action of *Withania somnifera* root extract. Indian Drugs 1993;30:305–12.

Kulkarni SK, Sharma A, Verma A, Ticku MK. GABA receptor mediated anticonvulsant action of *Withania somnifera* root extract. Indian Drugs 1993;30:305–12.

Kulkarni SK, George B, Nayar U. Amygdaloid kindling in rats protective effect of *Withania somnifera* (Ashwagandha) root extract. Indian Drugs 1994;32:37–49.

Kulkarni SK, George B, Mathur R. Neuroprotection by *Withania somnifera* root extract against lithium-pilocarpine-induced seizures. Indian Drugs 1998;35:208–15.

Kumar A, Kulkarni SK. Effect of BR-16A (Mentat) a polyherbal formulation on drug-induced catalepsy in mice. Indian J Exp Biol 2006;44:45–8.

Malhotra CL, Mehta VL, Das PK, Dhalla NS. Studies on Withania-ashwagandha Kaul V The effect of total alkaloids (ashwagandholine) on the central nervous system. Indian J Physiol Pharmacol 1965;9:127–36.

Matsuda H, Murakami T, Kishi A, Yoshikawa M. Structures of withanosides I II III IV V VI and VII new withanolide glycosides from the roots of Indian *Withania somnifera* DUNAL and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorg Med Chem 2001;9: 1499–507.

Mehta AK, Binkley P, Gandhi SS, Ticku MK. Pharmacological effects of *Withania somnifera* root extract on $GABA_A$ receptor complex. Indian J Med Res 1991;94:312–5.

Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha) a review. Altern Med Rev 2000;5: 334–46.

Mohan R, Hammers HJ, Bargagna-Mohan P, Zhan XH, Herbstritt CJ, Ruiz A, et al. Withaferin A is a potent inhibitor of angiogenesis. Angiogenesis 2004;7:115–22.

Mohanty I, Arya DS, Dinda A, Talwar KK, Joshi S, Gupta SK. Mechanisms of cardioprotective effect of *Withania somnifera* in experimentally induced myocardial infarction. Basic Clin Pharmacol Toxicol 2004;94:184–90.

Muruganandam AV, Kumar V, Bhattacharya SK. Effect of poly herbal formulation EuMil on chronic stress-induced homeostatic perturbations in rats. Indian J Exp Biol 2002;40:1151–60.

Nagashayana N, Sankarankutty P, Nampoothiri MR, Mohan PK, Mohanakumar KP. Association of L-DOPA with recovery following Ayurveda medication in Parkinson's disease. J Neurol Sci 2000;176:124–7.

Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on haloperidol-induced orofacial dyskinesia possible mechanisms of action. J Med Food 2003;6:107–14.

Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. Phytother Res 2006;20:140–6.

Olsen RW. The GABA postsynaptic membrane receptor-ionophore complex site of action of convulsant and anticonvulsant drugs. Mol Cell Biochem 1981;39:261–79.

Panda S, Kar A. Evidence for free radical scavenging activity of Ashwagandha root powder in mice. Indian J Physiol Pharmacol 1997;41:424–6.

Panda S, Kar A. Changes in thyroid hormone concentrations after administration of ashwagandha root extract to adult male mice. J Pharm Pharmacol 1998;50:1065–8.

Parihar MS, Hemnani T. Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. J Biosci 2003;28:121–8.

Parihar MS, Chaudhary M, Shetty R, Hemnani T. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice prevention by extracts of *Withania somnifera* and Aloe vera. J Clin Neurosci 2004;11:397–402.

Prabhu MY, Rao A, Karanth KS. Neuropharmacological activity of *Withania somnifera*. Fitoterapia LXI 1990;3:237–40.

Puri HS. Simple Ayurvedic Remedies. UBSPD, Delhi (India) (use of ashwagandha in various recipes); 2002.

Rai D, Bhatia G, Sen T, Palit G. Anti-stress effects of *Ginkgo biloba* and *Panax* ginseng a comparative study. J Pharmacol Sci 2003;93:458–64.

Rani G, Kaur K, Wadhwa R, Kaul SC, Nagpal A. Evaluation of the anti-genotoxicity of leaf extract of Ashwagandha. Food Chem Toxicol 2005;43:95–8.

Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. Phytother Res 1999;13:275–91.

Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine 2001;8:125–32.

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 2007;12:473–81.

Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. Neurochem Int 1997;30:181–90.

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 1986;23:133–9.

Shukla SD, Jain S, Sharma K, Bhatnagar M. Stress induced neuron degeneration and protective effects of *Semecarpus anacardium* Linn and *Withania somnifera* Dunn in hippocampus of albino rats an ultrastructural study. Indian J Exp Biol 2000;38:1007–13.

Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand KK. Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun. Phytother Res 2001;15:311–8.

Singh A, Naidu PS, Gupta S, Kulkarni SK. Effect of natural and synthetic antioxidants in a mouse model of chronic fatigue syndrome. J Med Food 2002:5211–20.

Sinha K, Degaonkar MN, Jagannathan NR, Gupta YK. Effect of melatonin on ischemia reperfusion injury induced by middle cerebral artery occlusion in rats. Eur J Pharmacol 2001;428:185–92.

Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. Life Sci 2002;71:655–65.

Stahl SM. Essential psychopharmacology. New Delhi Cambridge University Press; 1998.

Thakur RS, Puri HS, Hussain A. Major medicinal plants of India. CIMAP, Lucknow (India) (a monograph on *Withania somnifera*); 1987.

Ticku MK. Benzodiazepine-GABA receptor-ionophore complex: current concepts. Neuropharmacology 1983;22:1459–70.

Tohda C, Kuboyama T, Komatsu K. Dendrite extension by methanol extract of Ashwagandha (roots of *Withania somnifera*) in SK-N-SH cells. Neuroreport 2000;11:1981–5.

Tohda C, Kuboyama T, Komatsu K. Search for natural products related to regeneration of the neuronal network. Neurosignals 2005;14:34–45.

Vogel HG, Vogel WH, Scholkens BA, Sandow J, Muller G, Vogel WF. Pentylenetertazol (metrazol)-induced convulsions. Drug Discovery and Evalauation. Ed. 2nd. Berlin: Springer-Verlag; 2002. p. 422.

Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of Ashwagandha. J Ethnopharmacol 1996;50: 69–76.