Chapter – II

REVIEW OF RELATED LITERATURE

Medical researchers have been studying Ashwagandha with great interest and as of this date have carried out N number of studies of its medical prospective, healing benefits and psychomotor benefits, but only few attempts has been made by the researchers to explore its potential effect on sports performance in human. In this chapter researcher has tried to summarize the critical literature available till date on the effects of Ashwagandha supplementation on both animal and human.

Grandhi A et al. (1994) designed a comparative study between the pharmacological effects of Ashwagandha and Ginseng on rats and mice respectively. They conducted the tests comparatively between the aqueous suspensions of roots of an Indian drug Ashwagandha and the Korean drug Ginseng for 2 pharmacological activities, namely the anti-stress activity by the 'mice swimming endurance test' and anabolic activity by noting gain in body weights and levator ani muscle in rat. They found a significant increase in mice swimming time by giving them Ginseng (P < 0.001) and Ashwagandha (P < 0.01) as compared to the control group. They found that body weight was significantly increased in the Ashwagandha treated group (P < 0.05) and was better than Ginseng (P < 0.5). Gain in wet weights of the levator ani muscle were also significant in Ginseng (P < 0.001) and Ashwagandha (P < 0.01) treated groups, however, the weight gain of dried levator ani muscles showed comparable results for both these drugs (P < 0.01).

Grandhi A. et al. (1994) designed a comparative study between pharmacological activities of Indian drug Ashwagandha and the Korean drug Ginseng in rats and mice. They tested ashwagandha and ginseng comparatively for 2 pharmacological activities, namely the anti-stress activity by the 'mice swimming endurance test' and anabolic activity by noting gain in body weights
and levator ani muscle in rats. They found a significant increase in mice swimming time by treating with Ginseng (P < 0.001) and Ashwagandha (P < 0.01) as compared to the control group. They found body weights in the Ashwagandha treated group (P < 0.05) were better than Ginseng (P < 0.5) and gain in wet weights of the levator ani muscle were also significant in Ginseng (P < 0.001) and Ashwagandha (P < 0.01) treated groups but the weight gain of dried levator ani muscles showed comparable results for both these drugs (P < 0.01).

Ziauddin M et al. (1996) conducted a study to find out the immunomodulatory effects of Ashwagandha on mice. They studied the immunomodulatory activity of an Indian Ayurvedic medicinal preparation, Ashwagandha (Withania somnifera (L. Dunal)) in mice with myelosuppression induced by one or more of the following three compounds: cyclophosphamide, azathioprin, or prednisolone. They assessed the immunomodulatory activity was by hematological and serological tests. They found a significant modulation of immune reactivity in all the three animal models used. They found a significant increase in hemoglobin concentration (P < 0.01), red blood cell count (P < 0.01), white blood cell count (P < 0.05), platelet count (P < 0.01), and body weight (P < 0.05) in Ashwagandha-treated mice as compared with untreated (control) mice. They also found that treatment with Ashwagandha was accompanied by significant increases in hemolytic antibody responses towards human erythrocytes.

Schliebs R et al. (1997) conducted a study to assess the memory-enhancing effects of plant extracts from Withania somnifera and Shilajit and their affects on neurochemical alterations of specific transmitter systems in rat brain. Sitoindosides VII-X, and withaferin-A, isolated from aqueous methanol extract from the roots of cultivated varieties of Withania somnifera (known as Indian Ginseng), as well as Shilajit, a pale-brown to blackish brown exudation from steep rocks of the Himalaya mountain, are used in Indian medicine to attenuate cerebral functional deficits, including amnesia, in geriatric patients.
They conducted the present investigation to assess whether the memory-enhancing effects of plant extracts from Withania somnifera and Shilajit are owing to neurochemical alterations of specific transmitter system on the basis of the data collected they suggested that Shilajit and the defined extract from Withania somnifera affect preferentially events in the cortical and basal forebrain cholinergic signal transduction cascade. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition-enhancing and memory-improving effects of extracts from Withania somnifera observed in animals and humans.

Dhuley JN (1998) assessed the effect of ashwagandha on lipid peroxidation in stress-induced animals. They evaluated aqueous suspension of root extract of an Indian drug ashwagandha (Withania somnifera L. (Solanaceae) for its effect on lipid peroxidation (LPO) in stress-induced animals. They observed the elevation of LPO in rabbits and mice after intravenous administration of 0.2 microg/kg of lipopolysaccharide (LPS: from Klebsiella pneumoniae) and 100 microg/kg of peptidoglycan (PGN: from Staphylococcus aureus), respectively. They found the peak was reached immediately after PGN and 2-6 h after LPS administration. They further found that simultaneous oral administration of ashwagandha (100 mg/kg) prevented the rise in LPO in rabbits and mice.

Agarwal R et al. (1999) designed a study on mice to investigate immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation. They studied immunomodulatory activities of an Indian Ayurvedic medicinal preparation, i.e. extracts from Ashwagandha, Withania somnifera (L.) Dunal (Solanaceae), namely WST and WS2, in mice for immune inflammation. They assessed active paw anaphylaxis and delayed type hypersensitivity (DTH). They treated animals with WS2 at doses of 150 and 300 mg/kg, and compared the results with the standard drug disodium chromoglycate. They assessed the modulatory effect in the DTH model as potentiation or suppression of the reaction, revealing an increase or
decrease in mean foot pad thickness, respectively. They observed potentiation of the DTH reaction in animals treated with cyclophosphamide at a dose of 20 mg/kg, WST at a dose of 1000 mg/kg and WS2 at a dose of 300 mg/kg. On the other hand, they suppressed cyclophosphamide-induced potentiation of DTH reaction in animals treated with WST and WS2. They observed significant increase in white blood cell counts and platelet counts in animals treated with WST. They further observed a protective effect in cyclophosphamide-induced myelosuppression in animals treated with WST and WS2, revealing a significant increase in white blood cell counts and platelet counts.

**Bhattacharya et al. (2000)** examined the anxiolytic and antidepressant actions of the bioactive glycowithanolides (WSG), isolated from WS roots in rats. WSG(20 and 50 mg/kg) was administered orally once daily for 5 days and the results were compared by those elicited by the benzodiazepine lorazepam (0.5 mg/kg, i.p.) for anxiolytic studies, and by the tricyclic anti-depressant, imipramine (10 mg/kg, i.p.), for the antidepressant investigations. Both these standard drugs were administrated once, 30 min prior to the test. WSG induced an anxiolytic effect, comparable to that produced by lorazepam, in the elevated plus-maze, social interaction and feeding latency in an unfamiliar environment, tests. Further both WSG and lorazepam, rat brain levels of tribulin, an endocoid marker of clinical anxiety, when the levels were increased following administration of the anxoogenic agent. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced behavioural despair and learned helplessness tests. The investigations support the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in ayurveda.

**Singh et al. (2000)** explained the levels of corticosterone estimated by the HPCL method in the adrenal glands of stressed (5 h constant swimming) male albino mice treated with trichopus zeylanicus, Withania Somnifera and Panax ginseng preparations and compared them with non-treated stressed and normal controls in their study. The results of the study showed that increased
the corticosterone levels in all the groups, the physical endurance (increased survival time) of swimming mice also increased in all the treated groups, except in the group treated with Withania Somnifera powder (500 mg/kg, p.o).

**Bhattacharya SK et al. (2000)** designed a experimental study to investigate the anxiolytic and antidepressant actions of the bioactive glycowithanolides (WSG), isolated from WS roots, in rats. The roots of Withania Somnifera (WS) are used extensively in Ayurveda, the classical Indian system of medicine, and WS is categorized as a rasayana, which are used to promote physical and mental health, to provide defence against disease and adverse environmental factors and to arrest the aging process. WS has been used to stabilize mood in patients with behavioural disturbances. They administered WSG (20 and 50 mg/kg) orally once daily for 5 days and the results were compared by those elicited by the benzodiazepine lorazepam (0.5 mg/kg, i.p.) for anxiolytic studies, and by the tricyclic anti-depressant, imipramine (10 mg/kg i.p.), for the antidepressant investigations. They administered both these drugs once 30 min prior to the tests. WSG induced an anxiolytic effect, comparable to that produced by lorazepam, in the elevated plus-maze, social interaction and feeding latency in an unfamiliar environment, tests. Further, both WSG and lorazepam, reduced rat brain levels of tribulin, an endocoid marker of clinical anxiety, when the levels were increased following administration of the anxiogenic agent, pentylenetetrazole. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced 'behavioural despair' and 'learned helplessness' tests. On the basis of their investigations they supported the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda.

**Dhuley JN. (2000)** conducted a study to find the Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. They studied Pharmacological and metabolic effects of ashwagandha (Withania somnifera L. (Solanaceae)) used in Ayurveda as a herbal tonic and health food. Ashwagandha was shown to increase swimming time in rats in physical
working capacity test, i.e. rats swimming endurance test. They found significant increase in relative heart weight and glycogen content in myocardium and they observed liver also in ashwagandha treated group. They found ashwagandha treatment increased the duration of contractility in functional test for the resistance of frog heart muscle towards the toxic action of strophanthin-K. Ashwagandha treatment also resulted in significant increase in coagulation time which attains normalcy 7 days after cessation of treatment. They found ashwagandha did not possessed any toxicity up to a dose of (100 mg/kg; p.o. for 180 days) and did not caused significant changes in biochemical parameters in the blood serum of rats. On the basis of the observations they concluded that ashwagandha possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties

**Mishra LC et al. (2000)** in there critically reviewed the literature regarding Withania somnifera (ashwagandha, WS) a commonly used herb in Ayurvedic medicine system in India. Specifically, the literature was reviewed for articles pertaining to chemical properties, therapeutic benefits, and toxicity. They designed the review in a narrative format and consisted all publications relevant to ashwagandha that were identified by the authors through a systematic search of major computerized medical databases; they did not performed statistical pooling of results or evaluation of the quality of the studies due to the widely different methods employed by each study. On the basis of literature reviewed they found that studies indicates ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoietic, and rejuvenating properties. They said it also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems. They said that mechanisms of action for these properties are not fully known or under stoot yet. The studies revealed that ashwagandha did not have toxic effects and appeared to be a safe compound. They concluded that various constituents of ashwagandha exhibit a variety of therapeutic effects with little or no associated toxicity. They suggested that this herb should be studied more extensively to confirm these results and to reveal other potential
therapeutic effects. Clinical trials using ashwagandha for a variety of conditions should also be conducted.

Ilayperuma I et al. (2000) designed and conducted a study to know the effects of a methanolic extract of Withania somnifera roots on sexual competence of male rats. The 3000 mg./Kg/day dose of withania somnifera given to the male rats for 7 days. Their sexual behaviour was evaluated for 7 days prior to treatment, day 3 and 7 of treatment, 14 and 30 post-treatment by pairing each male with a receptive female. On the basis of results found they recommended that Withania somnifera roots may be detrimental to male sexual competence. The root extract induced a marked impairment in libido, sexual performance, sexual vigor and penile erectile dysfunction. These effects were partly reversible on cessation of treatment. They suggested that these masculine effects are not due to changes in testosterone levels or toxicity but may be attributed to hyper-prolactinemic, GABAergic, serotonergic or sedative activities of the extract.

Bucci L.R. et al. (2000) conducted a study to assess the effects of selected herbals on human exercise performance. In the literature they found the following herbs are currently used to enhance physical performance regardless of scientific evidence of effect: Chinese, Korean, and American ginsengs; Siberian ginseng, mahuang or Chinese ephedra; ashwagandha; rhodiola; yohimbe; CORDYCEPS: fungus, shilajit or mummio; smilax; wild oats; Muira puama; suma (ecdysterone); Tribulus terrestris; saw palmetto berries; beta-sitosterol and other related sterols; and wild yams (diosgenin). They found Asian ginseng improved the exercise performance when given for 8 weeks, daily dose 1 gram standardized root extract to a large number of subjects. They found improvements in muscular strength, maximal oxygen uptake, work capacity, fuel homeostasis, serum lactate, heart rate, visual and auditory reaction times, alertness, and psychomotor skills. Siberian ginseng showed mixed results. Mahuang, ephedrine, and related alkaloids did not benefited physical performance except when combined with caffeine. Other
herbs remained virtually untested. They suggested future research on ergogenic effects of herbs should consider identity and amount of substance or presumed active ingredients administered, dose response, duration of test period, proper experimental controls, measurement of psychological and physiologic parameters (including antioxidant actions), and measurements of performance pertinent to intended uses.

**Dhuley JN. et al. (2000)** assessed the adaptogenic and cardio-protective action of ashwagandha in rats and frogs. They studied the pharmacological and metabolic effects of ashwagandha (*Withania somnifera* L. (Solanaceae)) used in Ayurveda as a herbal tonic and health food were. They found that ashwagandha increased the swimming time in rats in physical working capacity test, i.e. rats swimming endurance test. They found significant increase in relative heart weight and glycogen content in myocardium and liver in ashwagandha treated group. Ashwagandha treatment increased the duration of contractility in functional test for the resistance of frog heart muscle towards the toxic action of strophanthin-K. They found ashwandha treatment also resulted in significant increase in coagulation time which attains normalcy 7 days after cessation of treatment. They further found that ashwagandha possesses no toxicity up to a dose of (100 mg/kg; p.o. for 180 days) and did not caused significant changes in biochemical parameters in the blood serum of rats. On the basis of the results found they concluded that ashwagandha possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties.

**Bhattacharya SK et al. (2000)** investigated the anxiolytic and antidepressant actions of the bioactive glycowithanolides (WSG), isolated from WS roots, in rats. The roots of *Withania somnifera* (WS) are used extensively in Ayurveda, the classical Indian system of medicine, and WS is categorized as a rasayana, which are used to promote physical and mental health, to provide defence against disease and adverse environmental factors and to arrest the aging process. WS has been used to stabilize mood in patients with behavioural
disturbances. They administered WSG (20 and 50 mg/kg) orally once daily for 5 days and compared the results by those elicited by the benzodiazepine lorazepam (0.5 mg/kg, i.p.) for anxiolytic studies, and by the tricyclic antidepressant, imipramine (10 mg/kg, i.p.), for the antidepressant investigations. They administered both these standard drugs once, 30 min prior to the tests. They found WSG induced an anxiolytic effect, comparable to that produced by lorazepam, in the elevated plus-maze, social interaction and feeding latency in an unfamiliar environment, tests. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced 'behavioural despair' and 'learned helplessness' tests. On the basis of investigations they supported the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda.

Abdel-Magied EM. (2001) conducted a study to assess the effects of aqueous extracts of Cynomorium coccineum and Withania somnifera on testicular development and on serum levels of testosterone, ICSH and FSH in immature Wistar rats. They found a notable increase in testicular weight of animals treated with both extracts. In the histological examination they found an apparent increase in the diameter of seminiferous tubules and the number of seminiferous tubular cell layers in the testes of treated rats as compared with control ones. They found extracts of both plants elicited notable spermatogenesis in immature rats but C. coccineum was more effective than W. somnifera in that respect and Serum testosterone and FSH levels were lower in animals treated with plants extracts than controls, whereas ICSH levels were higher in treated animals, specially in those treated with C. coccineum. They concluded that extracts of both plants have a direct spermatogenic influence on the seminiferous tubules of immature rats presumably by exerting a testosterone-like effect.

Dhuley JN. (2001) studied the Nootropic-like effect of ashwagandha (Withania somnifera L.) in mice. They administered Ashwagandha (Withania Somnifera L.) root extract (50, 100 and 200 mg/kg) orally for seven days and
found improved retention of a passive avoidance task in a step-down paradigm in mice. Ashwagandha (50, 100 and 200 mg/kg; orally) also 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. Daily administration of ashwagandha for 6 days significantly improved memory consolidation in mice receiving chronic ECS treatment. Ashwagandha, administered on day 7, also attenuated the disruption of memory consolidation produced by chronic treatment with ECS. They found ashwagandha reversed the scopolamine (0.3 mg/kg)-induced delay in transfer latency on day 1 on the elevated plus-maze. On the basis of their findings they suggested that ashwagandha exhibits a nootropic-like effect in naive and amnesic mice.

Ilayperuma I et al. (2002) determined the effect of a methanolic extract of Withania somnifera (L.) Dunal roots on sexual competence of male rats. They orally administered 3000 mg.kg-1.day-1 of root extract for 7 days to male rats and evaluated their sexual behaviour for 7 days prior to treatment, day 3 and 7 of treatment, and day 7, 14 and 30 post-treatment by pairing each male with a receptive female. They found the root extract induced a marked impairment in libido, sexual performance, sexual vigour, and penile erectile dysfunction. These effects were partly reversible on cessation of treatment. They found these antimasculine effects were not due to changes in testosterone levels or toxicity but may be attributed to hyperprolactinemic, GABAergic, serotonergic or sedative activities of the extract. On the basis of the results found they concluded that use of W. somnifera roots may be detrimental to male sexual competence.

Bhattacharya SK et al. (2003) conducted an experimental study to find out the antistress adaptogenic activity of Withania somnifera by using a rat model of chronic stress. Withania somnifera (WS) Dunal is classified in Ayurveda, the ancient Hindu system of medicine, as a rasayana, a group of plant-derived drugs reputed to promote physical and mental health, augment
resistance of the body against disease and diverse adverse environmental factors, revitalize the body in debilitated conditions and increase longevity. These attributes are remarkably similar to the properties ascribed to adaptogens like Panax ginseng (PG) in contemporary medicine. They investigated the adaptogenic activity of a standardised extract of WS roots against a rat model of chronic stress (CS). The stress procedure was mild, unpredictable footshock, administered once daily for 21 days to adult male Wistar rats. CS induced significant hyperglycaemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression. These CS induced perturbations were attenuated by WS (25 and 50 mg/kg po) and by PG(100 mg/kg po), administered 1 h before footshock for 21 days. On the basis of results they found indications that WS, like PG, has significant antistress adaptogenic activity, confirming the clinical use of the plant in Ayurveda.

Gupta SK et al. (2003) designed a study to investigate the effects of Withania somnifera on copper-induced lipid peroxidation and antioxidant enzymes in aging spinal cord of Wistar rats. Withania somnifera is classified in Ayurveda, the ancient Indian system of medicine, as a rasayana, a group of plant-derived drugs which promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalize the body in debilitated conditions and increase longevity. They found that treatment with Withania somnifera successfully attenuated GPx activity and inhibited lipid peroxidation in a dose dependent manner. Withania somnifera inhibited both the lipid peroxidation and protein oxidative modification induced by copper. These effects were similar to those of superoxide dismutase and mannitol. The results indicated the therapeutic potential of Withania somnifera in aging and copper-induced pathophysiological conditions.

Chaudhary G et al. (2003) investigated the effect of the Indian herbal plant Withania somnifera as a prophylactic treatment in the middle cerebral
artery (MCA) occlusion model of stroke in rats. 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. One of the approaches that can be used in limiting the neurological damage after stroke is the use of prophylactic treatment in patients with a high-risk of stroke. They conducted the present study to investigate the effect of the Indian herbal plant Withania somnifera as a prophylactic treatment in the middle cerebral artery (MCA) occlusion model of stroke in rats. They treated two groups of male Wistar rats with a hydroalcoholic extract of W. somnifera (1 g/kg, p.o.) for 15 and 30 days. Treatment with W. somnifera for 15 days could not improved motor performance or decreased the elevated levels of MDA. However, when the pre-treatment time of W. somnifera was increased to 30 days, it prevented motor impairment and significantly decreased the raised levels of MDA compared with vehicle-treated rats. They concluded W. somnifera has been documented to have anti-oxidant properties, the protection afforded by W. somnifera could be due to its anti-oxidant effect. The present study provides first evidence of the effectiveness of an Indian herb in focal ischaemia.

Iuvone T et al. (2003) studied the effect of Induction of nitric oxide synthase expression by Withania somnifera in macrophages. Withania somnifera (ashwagandha, Indian ginseng) is an immunostimulant herbal medicine used to improve overall health and prevent diseases, particularly in the elderly. However, the mechanisms underlying its immunostimulant effect is poorly understood. To elucidate the mechanism of Withania somnifera, they investigated the effect of a methanolic extract from the root of Withania somnifera (WS) on nitric oxide (NO) production in J774 macrophages. They found that WS (1-256 microg/ml) produced a significant and concentration-dependent increase in NO production, an effect which was abolished by N(G)nitro-L-arginine methyl ester (L-NAME, 3-300 microM), a non-selective inhibitor of NO synthase (NOS), dexamethasone (10 microM), an inhibitor of protein synthesis and N(alpha-p)-tosyl-L-lysine chloromethyl ketone (TLCK,
0.01-10 microM), an inhibitor of nuclear factor-kappaB (NF-kappaB) activation. They found Dexamethasone did not have any effect on NO production once NOS had been induced (i.e. 12 h after WS). Moreover, western blot analysis showed that WS increased, in a concentration-dependent fashion, inducible NOS protein expression. They concluded that WS may induce the synthesis of inducible NOS expression likely by acting at transcriptional level. The increased NO production by macrophages could account, at least in part, for the immunostimulant properties of Withania somnifera.

Singh B et al. (2003) examined adaptogenic activity of withanolide-free aqueous fraction from the roots of Withania somnifera Dun. They isolated a new withanolide-free hydrosoluble fraction from the roots of Withania somnifera Dun. and evaluated for putative antistress activity against a battery of tests to delineate the activity of this fraction. They found latter fraction exhibited significant antistress activity in a dose-related manner (Singh et al., 2001) and further studied chemical and physical induced stress in rats and mice. They used the extract of Withania somnifera root (a commercial preparation available locally) to compare the results. They found preliminary acute toxicity study in mice showed a good margin of safety with a high therapeutic index.

Parihar MS et al. (2004) examined the effects of combination of Withania somnifera and Aloe vera in prevention of susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice. Diabetes mellitus is reported to impair the memory function in experimental animals. They examined the vulnerability of cortex and hippocampus regions of the brain to oxidative damage in streptozotocin (STZ) diabetic mice and the attenuating effect of extracts of Withania somnifera and Aloe vera on prevention of hippocampal and cortical cell degenerations. Doses of both plant extracts given to experimental animals were based on the evaluation of their total antioxidant activity and also their potency to reduce Fe(3+). They assayed lipid peroxidation (LPO) and protein carbonyl (PC) in
both regions of the brain and observed the changes in memory and motor behavioral functions in diabetic and control mice. The results showed a significant (P < 0.05) increase in LPO and PC in hippocampus and cortical regions of STZ diabetic mice. They also found a significant impairment in both motor and memory behavioral functions in diabetic mice. However, when diabetic mice were supplemented with the extracts of Withania somnifera and Aloe vera, the oxidative damage in both brain regions was reduced as marked by a significant (p < 0.05) declines in both LPO and PC. The combination of extracts of Withania somnifera and Aloe vera was more effective in reducing oxidative damage in brain regions than the supplementation of single plant extract. The combination also lowered the blood glucose level in comparison to STZ diabetic mice. Memory impairment and motor dysfunction were also improved by the plant extracts supplementation. They concluded that impairments in the hippocampus and cortex in STZ diabetic mice are associated with an increased free radical mediated oxidative damage and that the supplementation of plant extracts showed preventive effects in attenuating oxidative damage in both brain regions possibly via antioxidative mechanisms.

Tohda C et al. (2005) designed a study to search the natural products related to regeneration of the neuronal network. The reconstruction of neuronal networks in the damaged brain is necessary for the therapeutic treatment of neurodegenerative diseases. They screened the neurite outgrowth activity of herbal drugs, and identified several active constituents. In each compound, neurite outgrowth activity was investigated under amyloid-beta-induced neuritic atrophy. Most of the compounds with neurite regenerative activity also demonstrated memory improvement activity in Alzheimer's disease-model mice. Protopanaxadiol-type saponins in Ginseng drugs and their metabolite, M1 (20-O-beta-D-glucopyranosyl-(20S)-protopanaxadiol), showed potent regeneration activity for axons and synapses, and amelioration of memory impairment. Withanolide derivatives (withanolide A, withanoside IV, and withanoside VI) isolated from the Indian herbal drug Ashwagandha, also showed neurite extension in normal and damaged cortical neurons. Trigonelline,
a constituent of coffee beans, demonstrated the regeneration of dendrites and axons, in addition to memory improvement.

Kuboyama T et al. (2005) investigated whether withanolide A (WL-A), isolated from the Indian herbal drug Ashwagandha (root of Withania somnifera), could regenerate neurites and reconstruct synapses in severely damaged neurons. They also investigated the effect of WL-A on memory-deficient mice showing neuronal atrophy and synaptic loss in the brain. Axons, dendrites, presynapses, and postsynapses were visualized by immunostaining for phosphorylated neurofilament-H (NF-H), microtubule-associated protein 2 (MAP2), synaptophysin, and postsynaptic density-95 (PSD-95), respectively. Treatment with A beta (25-35) (10 microM) induced axonal and dendritic atrophy, and pre- and postsynaptic loss in cultured rat cortical neurons. Subsequent treatment with WL-A (1 microM) induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre- and postsynapses in the neurons. WL-A (10 micromol kg (-1) day (-1), for 13 days, p.o.) recovered A beta (25-35)-induced memory deficit in mice. At that time, the decline of axons, dendrites, and synapses in the cerebral cortex and hippocampus was almost recovered. WL-A is therefore an important candidate for the therapeutic treatment of neurodegenerative diseases, as it is able to reconstruct neuronal networks.

Ahmad M et al. (2005) evaluated the anti-parkinsonian effects of Withania somnifera extract, which has been reported to have potent anti-oxidant, anti-peroxidative and free radical quenching properties in various diseased conditions. 6-Hydroxydopamine (6-OHDA) is one of the most widely used rat models for Parkinson's disease. They pretreated rats with 100, 200 and 300 mg/kg b.w. of the W. somnifera extract orally for 3 weeks. They infused 2 microL of 6-OHDA (10 microg in 0.1% in ascorbic acid-saline) on day 21 into the right striatum while sham operated group received 2 microL of the vehicle. They injected 6-OHDA to rats for three weeks and then tested their neurobehavioral activities. They found W. somnifera extract reversed all the
parameters significantly in a dose-dependent manner. On the basis of the findings they concluded that the extract of W. somnifera may be helpful in protecting the neuronal injury in Parkinson's disease.

**Bhatnagar M et al. (2005)** designed a comparative study to assess the Antiulcer and antioxidant activity of Asparagus racemosus Willd (Shatawari) and Withania somnifera Dunal with a standard drug, ranitidine, in various models of gastric ulcer in rats. They treated rats with A. racemosus as well as W. somnifera methanolic extract (100 mg/kg BW/day p.o.) orally for 15 days and found significant reduction in the ulcer index, volume of gastric secretion, free acidity, and total acidity. They also observed significant increase in the total carbohydrate and total carbohydrate/protein ratio. They concluded that A. racemosus was more effective in reducing gastric ulcer in indomethacin-treated gastric ulcerative rats, whereas W. somnifera was effective in stress-induced gastric ulcer. They compared results obtained for both herbal drugs to those of the standard drug ranitidine.

**Nagareddy PR et al. (2006)** designed a study to assess the effect of Withania somnifera (WS) root extract (ethanolic) for the improvement of bone clacification in calcium-deficient ovariectomized rats. Osteoporosis, characterized by reduction in bone density, is a significant source of mortality among the elderly, particularly in oestrogen-deficient women. They studied the effect of Withania somnifera (WS) root extract (ethanolic), which contains oestrogen-like withanolides for anti-osteoporotic activity. They treated female Sprague-Dawley rats with WS/vehicle (65 mg kg(-1)), orally for 16 weeks (n = 12). They allowed free access to a calcium-deficient diet (0.04% Ca) and distilled water to all rats. They measured urinary excretion of calcium (Ca) and phosphorus (P) and serum levels of Ca, P and alkaline phosphatase (ALP) after 16 weeks. They processed femur and tibia bones for histological (histology), morphological (scanning electron microscopy, SEM), biomechanical strength (impact test) and mineral composition (ash) analysis. On the basis of the results
found they concluded WS treatment markedly prevented the changes in OVX rats and thus may be a potential agent in the treatment of osteoporosis.

Naidu PS et al. (2006) conducted a study to investigate the effect of Withania somnifera root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. Tardive dyskinesia is one of the major side effects of long-term neuroleptic treatment. The pathophysiology of this disabling and commonly irreversible movement disorder is still obscure. Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia. Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of tardive dyskinesia. They treated rats with reserpine (1.0 mg/kg) on alternate days for a period of 5 days (days 1, 3 and 5) and found significantly induced vacuous chewing movements and tongue protrusions. They treated reserpine treated animals with Withania somnifera root extract (Ws) for a period of 4 weeks significantly and dose dependently (50 and 100 mg/kg) and found reduced reserpine-induced vacuous chewing movements and tongue protrusions. They found reserpine treated animals also showed poor retention of memory in the elevated plus maze task paradigm. Chronic Ws administration significantly reversed reserpine-induced retention deficits. On the basis of the findings of the study they indicated that oxidative stress might play an important role in the pathophysiology of reserpine-induced abnormal oral movements. They conclude that Withania somnifera root extract could be a useful drug for the treatment of drug-induced dyskinesia.

Mathur R et al. (2006) conducted a study to find out the effect of Withania somnifera root extracts on cell cycle and angiogenesis. They partitioned hydroalcoholic extract of the roots (WS) between chloroform (WS-chloroform) and water (WS-water). Further they fractionated WS-chloroform (A1-A12) by reverse-phase column chromatography and quantified their withanolide content by high-performance liquid chromatography (HPLC). Preliminarily, they screened anti-proliferative activity of all the extracts and fractions against human laryngeal carcinoma (Hep2) cells by microculture
tetrazolium assay (MTT). On the basis of the findings they suggested that the roots of Withania somnifera possess cell cycle disruption and anti-angiogenic activity, which may be a critical mediator for its anti-cancer action.

Kuboyama T et al. (2006) focused their study on exploring antidementia drug based on reconstructing neuronal networks in the damaged brain and found that withanoside IV (a constituent of Ashwagandha; the root of withania somnifera) induced neurite outgrowth in cultured rat cortical neurons. They found that oral administration of withanoside IV (10 micromol / kg / day) significantly improved memory deficits in mice and prevented loss of axons, dendrites, and synapses. They identified Sominone, an aglycone of withanoside IV as the main metabolite after oral administration of withanoside IV. On the basis of data collected they suggest that orally administrated withanoside IV may ameliorate neuronal dysfunction in Alzheimer’s disease and that the active principal after metabolism is sominone.

Naidu PS at el. (2006) examined the effect of withania somnifera root extract on reserpine-induced orofacacial dyskinesia and cognitive dysfunction. Tardive dyskinesia is one of the major side effects of long term neuroleptic treatment. The pathophysiology of this disabling and commonly irreversible movement disorder is still obscure. Vacuous chewing movements in rats are widely accepted as an animal mode of tardive dyskinesia. After chronic treatment with withania somnifera root extract for a period of 4 weeks with dose dependently (50 and 100 mg / kg) they found that reserpine-induced vacuous chewing movements and tongue protrusions reduced. Reserpine treated animals also showed poor retention of memory in the elevated plus maze task paradigm. They found chronic withania somnifera administration significantly reversed reserpine-induced retention deficits. Chronic reserpine treated rats showed decreased levels of antioxidant defense enzymes, superoxide dismutase (SOD) and catalase. They found chronic administration of withania somnifera root extract dose dependently (50 and 100 mg / kg) significantly reduced the lipid peroxidation and restored the decreased
glutathione levels by chronic reserpine treatment. The major finding of the present study indicates the oxidative stress might play an important role in the pathophysiology of reserpine-induced abnormal oral movements. On the basis of their findings they concluded that withania somnifera root extract can be a useful drug for the treatment of drug-induced dyskinesia.

Shah PC et al. (2006) investigated the effect of Withania Somnifera on forced swimming test induced immobility in mice and its interaction with various drugs. They started with the objective to evaluate the antidepressant action of Withania somnifera (WS) as well as its interaction with the conventional antidepressant drugs and to delineate the possible mechanism of its antidepressant action using forced swimming model in mice. They studied the effect of different doses of WS, fluoxetine and imipramine on forced swimming test induced mean immobility time (MIT). They observed effect of WS 100 mg/kg, i.p. at different time intervals. They observed effect produced by combination of sub therapeutic doses of WS with imipramine (2.5 mg/kg, i.p.) as well as fluoxetine (2.5 mg/kg, i.p.). They observed effect of WS (100 mg/kg, i.p.) as well as combination of WS (37.5 mg/kg, i.p.) with either imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) in mice pre-treated with reserpine (2 mg/kg, i.p.) and clonidine (0.15 mg/kg, i.p.). Effects of prazosin (3 mg/kg, i.p.) or haloperidol (0.1 mg/kg, i.p.) pre-treatment were also observed on WS induced decrease in MIT. Maximum effect in MIT was observed after 30 min of treatment with WS 100 mg/kg, i.p. Combination of WS (37.5 mg/kg, i.p.) with imipramine (2.5 mg/kg, i.p.) or fluoxetine (2.5 mg/kg, i.p.) also produced significant decrease in the MIT. On the basis of results found they concluded that, WS produced significant decrease in MIT in mice which could be mediated partly through a adrenoceptor as well as alteration in the level of central biogenic amines.

Gupta GL et al. (2007) examined the effects of Withania Somnifera Dunal (WS) root extract and diazepam in social isolation induced behavior such as anxiety and depression in rats. They isolated the rats for 6 weeks and
observed the assessment of changed behavior on elevated plus maze (EPM) and forced swim test (FST). They found Isolation reared rats spent less time into the open arms on EPM and significantly increased immobility time in FST compared to group housed rats. WS (100, 200 or 500 mg/kg, oral) and diazepam (1 or 2 mg/kg, ip) dose dependently increased the time spent and entries into the open arms on EPM test and showed the anxiolytic activity. Sub effective dose of WS (50 mg/kg, oral) potentiated the anxiolytic action of diazepam (0.5, 1 or 2 mg/kg, ip). WS (100, 200 or 500 mg/kg, oral) also reduced the immobility time in FST, thus showed antidepressant effect in both group housed and social isolates. On the basis of the investigations and results found they supported the use of WS as a mood stabilizer in socially isolation behavior in Ayurveda.

Rasool M et al. (2007) conducted a study to evaluate protective effect of Withania somnifera root powder commonly known as Ashwagandha in relation to lipid peroxidation, antioxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. They induced Arthritis by intradermal injection of complete Freund's adjuvant (0.1 mL) into the right hind paw of Wistar albino rats. They administered Withania somnifera root powder (1000 mg/kg/day) and Indomethacin (3 mg/kg/day) orally for 8 days (from 11th to 18th day) after adjuvant injection. They assessed anti-arthritic effect of W. somnifera root powder by measuring changes in lipid peroxidation, antioxidant status, and glycoprotein levels in plasma and spleen of arthritic animals. They assessed cartilage degradation also by estimating bone collagen, and urinary constituents in arthritic animals. They found significant increase in the level of lipid peroxides, glycoproteins, and urinary constituents with the depletion of antioxidant status and bone collagen in arthritic animals. These biochemical alterations observed were ameliorated significantly by oral administration of W. somnifera root powder (1000 mg/kg body weight) in arthritic animals. They concluded that W. somnifera root powder is capable of rectifying the above biochemical changes in adjuvant arthritis.
**Gupta GL et al. (2007)** investigated the effect of Withania somnifera Dunal (WS) root extract and diazepam in social isolation induced behavior such as anxiety and depression in rats. They isolated rats for 6 weeks and assessed the changed behavior on elevated plus maze (EPM) and forced swim test (FST). They found Isolation reared rats spent less time into the open arms on EPM and significantly increased immobility time in FST compared to group housed rats. On the basis of the results found they supported the use of WS as a mood stabilizer in socially isolation behaviour in Ayurveda.

**Kumar A et al. (2007)** investigated the protective effect of W. somnifera on the behavioral and biochemical alterations in sleep disturbed mice. Sleep disruption involves extensive changes in physiological function, including EEG, motor, metabolic, autonomic processes physiological homeostasis and psychological balance that are necessary for physical health. Benzodiazepines are the most widely used drugs for the sleep related problems in spite of their limitations and side effects. Objective of the study was to investigate the protective effect of W. somnifera on the behavioral and biochemical alterations in sleep disturbed mice. They found treatment with W. somnifera root extract (100, 200 mg/kg) and diazepam (0.5 mg/kg) significantly protected reduction in body weight, improved the reduced locomotor activity and anxiety levels in animals. Biochemical studies also revealed that W. somnifera (100 and 200 mg/kg) and diazepam (0.5 mg/kg) pretreatment for five days decreased significantly lipid peroxidation, nitrites levels and improved catalase, and reduced glutathione levels. They found co-administration of W. somnifera (100 mg/kg) with diazepam (0.5 mg/kg) improved significantly all the biochemical parameters as compared to their effect per se. On the basis of results they suggested that Withania root extract can be used in the management sleep loss and associated oxidative stress.

**Krishnamurthy MN et al. (2007)** conducted a study to assess the effect of two ancient Indian interventions: effects of yoga and ayurveda on older adults in a residential homes with depressive symptoms. They evaluated the
effects of yoga and ayurveda on geriatric depression in 69 persons older than 60 who were living in a residential home. They stratified participants by age and gender and randomly divided them in three groups: Yoga, Ayurveda, or Wait-list Control. They used 15-item Geriatric Depression Scale to assess depressive symptoms prior to the intervention, and after 3 months and 6 months post-intervention. The yoga program (7 hours 30 minutes per week) included physical postures, relaxation techniques, regulated breathing, devotional songs, and lectures. The Ayurveda Group given an herbal preparation twice daily for the whole period. They found depression symptom scores of the Yoga Group at both 3 and 6 months decreased significantly, from a group average baseline of 10.6 to 8.1 and 6.7, respectively (p < .001, paired t-test). The other groups showed no change. On the basis of results they recommended an integrated approach of yoga including the mental and philosophical aspects in addition to the physical practices is useful for institutionalized older persons.

Tohda C (2008) focused in his review on the effects of compounds isolated from Ashwagandha on dementia models and on the spinal cord injury model. He administered orally Withanolide A, Withanoside IV, and Withanoside V, (10 micromol/ kg/ day for 12 days) and found improvement in induced memory impairment, neurite atrophy, and synaptic loss in the cerebral cortex and hippocampus in mice. They treated mice with Withanoside IV 10 micromol/ kg/ day for 21 days, and found axonal density and peripheral nervous system myelin level increased. The loss of CNS myelin and increase in reactive gliosis were not affected by Withanoside IV. Additionally, sominone, an aglycone of Withanoside IV, was identified as the main metabolite after oral administration of Withanoside IV in mice. On the basis of his findings he concluded that Withanolide A, Withanolide IV, and Withanoside VI are important candidates for the therapeutic treatment of neurodegenerative diseases.
Kulkarni SK et al. (2008) conducted a study to find out the effect of Withaniasomnifera root extract (Ws) alone or in combination with exogenous gamma-aminobutyric acid (GABA), a GABA receptor agonist or with diazepam, a GABA receptormodulator against pentylenetetrazol (PTZ, iv) seizure threshold in mice. Minimal dose of PTZ (iv, mg/kg) needed to induce different phases (myoclonic jerks, generalized clonus and tonic extension) of convulsions were recorded as an index of seizure threshold. They found Ws (100 or 200 mg/kg, po) increased the PTZ seizure threshold for the onset of tonic extension phase whereas a lower dose (50 mg/kg, po) did not show any effect on the seizure threshold. Co-administration of a sub-effective dose of Ws (50 mg/kg, po) with a sub-protective dose of either GABA (25 mg/kg, ip) or diazepam (0.5 mg/kg, I p) increased the seizure threshold. On the basis of the results they suggested that the anticonvulsant effect of W. somnifera against PTZ seizure threshold paradigm involved the GABAAergic modulation.

Tohda C. et al. (2008) conducted a study to find out the treatment of several neurodegenerative diseases by traditional medicines, the development of therapeutic medicines and to understand their unraveling pathophysiological mechanisms. Ashwagandha (root of Withania somnifera) has been used for many purposes, it is mainly considered a tonic in traditional Ayurvedic medicine. In this review they focused on the effects of compounds isolated from Ashwagandha on dementia models and on the spinal cord injury model. In their study they demonstrated that the active constituents, withanolide A, withanoside IV, and withanoside VI, restored presynapses and postsynapses, in addition to both axons and dendrites in cortical neurons after Abeta(25-35)-induced injury. They found oral administration of withanolide A, withanoside IV, and withanoside VI (10 micromol/kg/day for 12 days) improved Abeta(25-35)-induced memory impairment, neurite atrophy, and synaptic loss in the cerebral cortex and hippocampus in mice. They found oral treatment with withanoside IV improved locomotor functions in mice with SCI and mice treated with withanoside IV (10 micromol/kg/day for 21 days), the axonal density and peripheral nervous system myelin level increased. The loss of CNS
myelin and increase in reactive gliosis were not affected by withanoside IV. They identified sominone, an aglycone of withanoside IV, as the main metabolite after oral administration of withanoside IV in mice. According to their findings they concluded that Withanolide A, withanoside IV, and withanoside VI are important candidates for the therapeutic treatment of neurodegenerative diseases.

Gupta GL. et al. (2008) designed and conducted a study to assess the effect of Withania somnifera Dunal in ethanol-induced anxiolysis and withdrawal anxiety in rats. Withania somnifera (WS) or its psychotropic preparation is known to play a critical role in morphine, alcohol and benzodiazepines addiction. They investigated the role of WS in acute ethanol and withdrawal from chronic ethanol consumption using elevated plus maze paradigm in rats. They found acute administration of ethanol (1.5-2 g/kg, ip) triggered anxiolytic effect and withdrawal from prolonged ethanol (9% v/v ethanol, 15 days) consumption elicited enhanced behavioral despair (anxiety). Acute administration of WS (50 mg/kg, oral) potentiated the anxiolytic action of sub-effective dose of ethanol (0.5 or 1 g/kg, ip). Moreover, the ethanol withdrawal anxiety was markedly antagonized in dose dependent manner by WS at 200 and 500 mg/kg or higher dose of ethanol (2.5 g/kg). However, They found co-administration of sub effective doses of WS (50 mg/kg, oral) and ethanol also attenuated withdrawal-induced anxiety due to chronic ethanol (9% v/v ethanol, 15 days) consumption. On the basis of their findings they suggested that WS shows protective effects in the management of ethanol withdrawal reactions.

Kulkarni SK. et al. (2008) designed a study to assess the effect of some anti anxiety drugs on behavior of different mazes. Anxiety is associated with diverse range of psychiatric conditions. They prepared a comparative behavioural profile to study the changes. They examined the anti anxiety effects of fluoxetine, citalopram (SSRI's), gabapentin (antiepileptic drugs), venlafaxine (SNRI), clozapine and resperidone (atypical antipsychotics) and a
herbal preparation ashwagandha on elevated zero maze and elevated plus maze paradigms. They compared the anti-anxiety potentials of these drugs with diazepam. They tested a couple of drugs i.e. fluoxetine (10 mg/kg), citalopram (10 mg/kg), clozapine (0.25, 0.5, 1 mg/kg), resperidone (0.5, 1 mg/kg), venlafaxine (4, 8, 16 mg/kg), citalopram (10 mg/kg), fluoxetine (10 mg/kg), gabapentin (10, 20 mg/kg) and ashwagandha (100, 200 mg/kg) and found that the drugs significantly increased the number of open arm entries and time spent in open arm. These drugs also decreased the latency to enter in open arm as compared to control in both the paradigms. In the conclusion they confirmed the anti anxiety activity of different newer classes of drugs and found some of them comparable to diazepam in both the elevated zero maze and elevated plus maze paradigm.

Kasture S et al. (2009) designed and conducted a study to investigate the effects of Withania somnifera in prevention of morphine withdrawal-induced decrease in spine density in nucleus accumbens shell of rats. Opiate withdrawal is associated with morphological changes of dopamine neurons in the ventral tegmental area and with reduction of spine density of second-order dendrites of medium size spiny neurons in the nucleus accumbens shell but not core. Withania somnifera has long been used in the Middle East, Africa, and India as a remedy for different conditions and diseases and a growing body of evidence points to its beneficial effects on a number of experimental models of neurological disorders. In this study they investigated whether morphine withdrawal-induced spine reduction in the nucleus accumbens is affected by the administration of a Withania somnifera extract. They treated rats with Withania somnifera extract along with morphine or saline and, upon spontaneous (1 and 3 days) or pharmacologically precipitated withdrawal, they found their brains were fixed in Golgi-Cox stain for confocal microscopic examination. They administered Withania somnifera extract in a separate group of animals, during three days of spontaneous withdrawal. They found withania somnifera extract treatment reduced the severity of the withdrawal syndrome when given during chronic morphine but not during withdrawal. On the basis
of the results they concluded that pretreatment with Withania somnifera extract protects from the structural changes induced by morphine withdrawal potentially providing beneficial effects on the consequences related to this condition.

Jeyanthi T et al. (2009) investigated the protective effect of Withania somnifera, an indigenous medicinal herb used in ayurvedic traditional systems on gentamicin (GEN)-induced nephrotoxicity. They administered root extract of three different doses of W. somnifera (viz., 250, 500, and 750 mg/kg) orally to rats for 14 days before GEN treatment and thereafter concurrently with GEN (100 mg/kg) for 8 days. They found significant increase in kidney weight, urea, creatinine, urinary protein, and glucose, and significant reduction in body weights and potassium in GEN-treated rats, which they confirmed histopathologically by tubular necrosis. They found in contrast W. somnifera (500 mg/kg) significantly reversed these changes as evidenced microscopically when compared to other two doses of W. somnifera (250 and 750 mg/kg), and there were no significant changes in the levels of sodium in the experimental animals compared to control. On the basis of the results found they supported the nephroprotective effect of Withania somnifera, which could be by enhancing antioxidant activity with natural antioxidants and scavenging the free radicals.

Shah N H et al. (2009) conducted a study to know the effects of alcoholic extracts of Ashwagandha leaves and its components on proliferation, migration, and differentiation of glioblastoma cells. Ashwagandha (Withania somnifera) is widely used in the Indian traditional system of medicine, Ayurveda. Although it is claimed to have a large variety of health-promoting effects, including therapeutic effects on stress and disease, the mechanisms of action have not yet been determined. In the present study, they investigated the growth inhibition and differentiation potential of the alcoholic extract of Ashwagandha leaves (i-Extract), its different constituents (Withaferin A, Withanone, Withanolide A) and their combinations on glioma (C6 and YKG1)
cell lines. On the basis of the data collected they suggested that the i-Extract and its components have the potential to induce senescence-like growth arrest and differentiation in glioma cells.

Kumar P et al. (2009) investigated the effects of Withania somnifera root extract against 3-NP-induced gait abnormalities, oxidative stress, and mitochondrial dysfunction in striatum and cortex of rat brain. Huntington's disease (HD) is a neurodegenerative disorder that results from the destruction of neurons in the basal ganglia, and oxidative stress has been implicated in its pathogenesis. 3-Nitropropionic acid (3-NP), a potent neurotoxin, has been reported to induce oxidative/nitrosative stress and causes neurobehavioral and biochemical changes that mimic HD in humans. It also inhibits complex II of the mitochondrial electron transport chain, thereby causing cellular energy deficit. They evaluated the effects of a well-known antioxidant on behavioral, biochemical, and mitochondrial dysfunction induced by 3-NP. They investigated the effects of Withania somnifera root extract against 3-NP-induced gait abnormalities, oxidative stress, and mitochondrial dysfunction in striatum and cortex of rat brain. They found Intraperitoneal administration of 3-NP (10 mg/kg for 14 days) caused a loss in body weight and a decline in motor function (locomotor activity and impaired rotarod activity). Chronic treatment with W. somnifera root extracts (100 and 200 mg/kg) for a period of 2 weeks dose-dependently improved 3-NP-induced behavioral, biochemical, and enzymatic changes (P < .05). Biochemical analysis revealed that systemic 3-NP administration significantly increased lipid peroxidation and nitrite and lactate dehydrogenase enzyme levels, depleted antioxidant enzyme (superoxide dismutase and catalase) levels, and blocked ATP synthesis by inhibiting the mitochondrial complex activity in the different regions (striatum and cortex) of the brain. Chronic administration of W. somnifera root extract (100 and 200 mg/kg) dose-dependently restored biochemical alterations induced by chronic 3-NP treatment (P < .05). On the basis of the findings they suggested that neuroprotective actions of W. somnifera are mediated via its antioxidant activity. However, further studies are required to elucidate the molecular
mechanisms involved in order to support the clinical use of the plant extract as a therapeutic agent for the treatment of HD.

**Tohda C et al. (2009)** assessed the effect of Sominone an aglycone of withanoside iv (a compound isolated from the roots of Withania somnifera) on neurite outgrowth and spatial memory mediated by the neurotrophic factor receptor, RET. They aimed to identify receptors or associated molecules of sominone, and to investigate the effects of sominone on memory in normal mice. They compared Phosphorylation levels of 71 molecules between control and sominone-stimulated cortical cultured cells to search for target molecules of sominone. Object location memory and neurite density in the brain were evaluated in sominone-injected mice. On the basis of the results they found that phosphorylation of RET (a receptor for the glial cell line-derived neurotrophic factor, GDNF) was increased in neurons by sominone, without affecting the synthesis and secretion of GDNF. Knockdown of RET prevented sominone-induced outgrowths of axons and dendrites. After a single i.p. injection of sominone into normal mice, they could better memorize scenery information than control mice. Sixty minutes after sominone injection, RET phosphorylation was increased, particularly in the hippocampus of mice. After the memory tests, the densities of axons and dendrites were increased in the hippocampus by sominone administration. They concluded that Sominone could reinforce the morphological plasticity of neurons by activation of the RET pathway and thus enhance memory. Sominone, a compound with low molecular weight, may be a GDNF-independent stimulator of the RET pathway and/or a novel modulator of RET signalling.

**Kasture S et al. (2009)** conducted a study to find out the effect of Caffeine withdrawal retains and Withania Somnifera withdrawal on haloperidol-induced catalepsy in mice. The previous studies available showed that chronic treatment with Withania somnifera extract (WS) inhibited haloperidol-induced catalepsy and suggested that caffeine and WS may be useful adjuvants in pharmacotherapy of Parkinson's disease. No studies found
on the effect of haloperidol on mice withdrawn from caffeine or W. somnifera. They therefore studied the effect of a single administration of standardised WS containing 5.1% total withanolides (WS, 30 or 100 mg kg (-1) i.p.) and/or caffeine (3 mg kg(-1) i.p.) and withdrawal from 6 days treatment with WS and/or caffeine, on haloperidol-induced catalepsy in albino mice. They found that single administration of both WS and caffeine, used either alone or in combination, significantly inhibited catalepsy. Mice withdrawn from caffeine significantly inhibited haloperidol-induced catalepsy, but mice withdrawn from WS showed increased catalepsy. On the basis of the results found they concluded that withdrawal from WS does not retain anticataleptic activity, and caffeine but not WS may be a good adjuvant in pharmacotherapy of Parkinson's disease.

Raja Sankar S et al. (2009) examined the effect of Ws on catecholamines and physiological abnormalities seen in Parkinson's disease (PD) using Parkinson's disease (PD) model mouse. Withania somnifera root extract (Ws)/Ashwagandha/Indian ginseng is a traditional herbal medicine, used over 4000 years in India, shown to have effect on neural growth and locomotor function. Although catecholamines and oxidative stress resulting in neurodegeneration and locomotor disorder are the main events in Parkinson's disease (PD), efficacy of the drug on these molecules and physiological abnormality are not clear. They treated mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for 4 days to show biochemical and physiological abnormalities similar to patients with PD. PD mice were treated with Ws 100mg/kg body weight for 7 or 28 days. Catecholamines: dopamine (DA), 3,4-dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid(HVA); antioxidants: glutathione (GSH) and glutathione peroxidase (GPx); and lipid peroxidation marker (TBARS) were analyzed in the Ws treated and untreated PD mouse striatum. They found mouse treated with MPTP showed reduced levels of DA, DOPAC, HVA, GSH and GPx and induced thiobarbituric acid reactive substance (TBARS) level compared to the control. Physiological abnormalities were seen in the mouse as determined by hang test and rotarod
test. Oral treatment of PD mouse Ws root extract (100mg/kg body weight) for 7 days or 28 days increased DA, DOPAC and HVA levels and normalized TBARS levels in the corpus striatum of the PD mouse. The 7 days Ws treated mice showed improved motor function as determined by hang test and rotarod test. Treatment with Ws for 28 days increased GSH and GPx levels in the striatum compared to the Ws untreated PD mouse striatum. On the basis of data collected they concluded that Ws is a potential drug in treating catecholamines, oxidative damage and physiological abnormalities seen in the PD mouse.

Jayaprakasam B et al. (2010) tested the ability of Withanamides in Withania fruit to protect the PC-12 cells from beta – amyloid responsible for Alzheimer’s disease. Alzheimer’s disease (AD) is an irreversible neurodegenerative disorder with symptoms of confusion, memory loss, and mood swings. The beta-amyloid peptide, with 39-42 amino acid residues (BAP), plays a significant role in the development of AD. Although there is no cure for AD, it can be managed with available drugs to some degree. Several studies have revealed that natural antioxidants, such as vitamin E, vitamin C and beta-carotene, may help in scavenging free radicals generated during the initiation and progression of this disease. In this study, they tested two major withanamides A (WA) and C (WC) for their ability to protect the PC-12 cells, rat neuronal cells, from beta-amyloid induced cell damage. They found the cell death caused by beta-amyloid was negated by withanamide treatment. They further found that withanamide A and C uniquely bound to the active motif of beta-amyloid (25-35) and suggests that withanamides have the ability to prevent the fibril formation. Further understanding of the mechanism of action and in vivo efficacy of these withanamides may facilitate its development as prophylaxis.

Kumar S et al. (2010) investigated the protective effects of Withania somnifera (L.) dunal root extract against hydrogen peroxide and β-amyloid (1-42)-induced cytotoxicity in differentiated PC12 cells. Withania somnifera L. Dunal (Solanaceae), also known as 'ashwagandha' in Sanskrit and as 'Indian
ginseng', is used widely in Ayurvedic medicine as a nerve tonic and memory enhancer, with antiaging, antistress, immunomodulatory and antioxidant properties. There is a paucity of data on the potential neuroprotective effects of W. somnifera root, as traditionally used, against H(2)O(2)- and Aβ((1-42))-induced cytotoxicity which are current targets for novel approaches to treat dementia, especially dementia of the Alzheimer's type (AD). In this study, they prepared an aqueous extract from the dried roots of W. somnifera and assessed for potential protective effects against H(2)O(2)- and Aβ((1-42))-aggregated fibrilcytotoxicity by an MTT assay using a differentiated rat pheochromocytoma PC12 cell line. On the basis of results found they suggested that pretreatments of differentiated PC12 cells with aqueous extracts of W. somnifera root significantly protect differentiated PC12 cells against both H(2)O(2)- and Aβ((1-42))-induced cytotoxicity, in a concentration dependent manner. To investigate the compounds that could explain the observed effects, the W. somnifera extract was analysed by liquid chromatography-serial mass spectrometry and numerous withanolide derivatives, including withaferin A, were detected. These results demonstrate the neuroprotective properties of an aqueous extract of W. somnifera root and may provide some explanation for the putative ethnopharmacological uses of W. somnifera for cognitive and other neurodegenerative disorders that are associated with oxidative stress.

Shah JS et al. (2010) explored the trends and rationale of use of memory and vitality-enhancing medicines (MVEM) in the Gujarat region. A prospective pharmacoepidemiological study involving pharmacists of Gujarat region was carried out in the year 2005. 351 pharmacists working in general and Ayurvedic medical stores were selected from 12 districts of Gujarat region. The pharmacists were explained about the objective of the study and were given a pretested, validated questionnaire. The questionnaire included the questions regarding herbal MVEM used most commonly, percentage sale of herbal MVEM - sold with or without prescriptions, age group of patients and professional groups who used these drugs most commonly. They found the
number of individuals using MVEM was highest in the age group of 11-20 years (17.54%), followed by the 21-40 years group (17.12%), supporting the results that the professional group of students (17.29%) and the persons of business or service class (15.29%) are the highest users of these medicines. Evaluation of various constituents in the marketed polyherbal MVEM revealed that Brahmi (Bacopa monniera), Shankhpushpi (Evolvulus alsinoides), Ashwagandha (Withania somnifera), Jatamansi (Nardostychos jatamansi), Vacha (Acorus calamus) and Amla (Phyllanthus emblica) were the common ingredients in the polyherbal preparations. They concluded that commonly used Ayurvedic medicines can be explored for safely enhancing memory and vitality performance. Hence, detailed and scientifically designed research on these drugs would help to identify safe and effective drugs for enhancing the same.

Kumar S et al. (2010) investigated the effects of W Somnifera root, as traditionally used, against H(2)O(2)- and Aβ ((1-42))-induced cytotoxicity which are current targets for novel approaches to treat dementia, especially dementia of the Alzheimer's type (AD). Withania somnifera L. Dunal (Solanaceae), also known as 'ashwagandha' in Sanskrit and as 'Indian ginseng', is used widely in Ayurvedic medicine as a nerve tonic and memory enhancer, with anti aging, antistress, immunomodulatory and antioxidant properties. In this study, they prepared an aqueous extract from the dried roots of W. somnifera and assessed for potential protective effects against H(2)O(2)- and Aβ((1-42))-aggregated fibril cytotoxicity by an MTT assay using a differentiated rat pheochromocytoma PC12 cell line. On the basis of the results they suggested that pretreatments of differentiated PC12 cells with aqueous extracts of W. Somnifera root significantly protect differentiated PC12 cells against both H(2)O(2)- and Aβ((1-42))-induced cytotoxicity, in a concentration dependent manner. To investigate the compounds that could explain the observed effects of W. Somnifera extract they analysed Withania somnifera by liquid chromatography-serial mass spectrometry and found numerous withanolide derivatives, including withaferin A. Their results demonstrate the neuroprotective properties of an aqueous extract of W. Somnifera root and may
provide some explanation for the putative ethnopharmacological uses of W. Somnifera for cognitive and other neurodegenerative disorders that are associated with oxidative stress.

**Sandhu JS et al. (2010)** conducted a scientific clinical study showing effect of Withania somnifera (Ashwagandha) and Terminalia arjuna (Arjuna) both these drugs on exercise performance after regular administration when given as supplements The study was therefore designed and performed to assess the effects of Ashwagandha and Arjuna individually and as a combination on maximum velocity, average absolute and relative Power, balance, maximum oxygen consumption (VO2 max) and blood pressure in humans. Forty normal healthy. Subjects (either sex, mean age 20.6 ± 2.5yrs and mean Body Mass Index 21.9 ± 2.2) were recruited after written informed consent was obtained. Institutional Ethics Committee permission was also obtained. Thirty participants were assigned to experimental group of which 10 received standardized root extracts of Withania somnifera, 10 received standardized bark extract of Terminalia arjuna and the rest of the 10 received standardized root extract of Withania somnifera in addition to bark extract of Terminalia arjuna both. Both the drugs were given in the form of capsules (dosage 500mg/day for both the drugs). Ten participants received placebo (capsules filled with flour). All the subjects continued the regimen for 8 weeks. All variables were assessed before and after the course of drug administration. Withania somnifera increased velocity, power and VO2 max whereas Terminalia arjuna increased VO2 max and lowered resting systolic blood pressure. When given in combination, the improvement was seen in all parameters except balance and diastolic blood pressure. Withania somnifera may therefore be useful for generalized weakness and to improve speed and lower limb muscular strength and neuro-muscular co-ordination. Terminalia arjuna may prove useful to improve cardio-vascular endurance and lowering systolic blood pressure. Both drugs appear to be safe for young adults when given for mentioned dosage and duration.
Pawar P et al. (2011) investigated the anti-inflammatory and muco-restorative activity in TNBS-induced inflammatory bowel disease in rats by rectal gel application of Withania somnifera root extract. Inflammatory Bowel Disease (IBD) is marked with chronic inflammation of intestinal epithelium driven by oxidative stress. Traditional treatments with plant extracts gained renewed interest due to their ability to ameliorate the multi factorial conditions like inflammation. They investigated the beneficial effects of Withania somnifera in Trinitro Benzyl Sulfonic Acid (TNBS) induced experimental IBD through a rectally applicable formulation. They prepared gel formulation from aqueous Withania somnifera root extract (WSRE). They found WSRE treatment positively scored on histopathological parameters like necrosis, edema, neutrophil infiltration and the post treatment intestinal features showed restoration at par with the healthy intestine. In view of these results, they tested gel formulation containing an aqueous extract of W. somnifera, prepared for rectal application for its anti-inflammatory activity in TNBS-induced rat models for IBD. They used commercially available anti-inflammatory drug Mesalamine as the standard in this assay. On the basis of the results they found that dose of the rectal gel applied at 1000 mg of WSRE per kg rat weight showed significant muco-restorative efficacy in the IBD-induced rats, validated by histo-pathological studies.

Ramanathan M et al. (2011) conducted a study to study the Behavioural and neurochemical evaluation of Perment an herbal formulation in chronic unpredictable mild stress induced depressive model. Perment is a polyherbal Ayurvedic formulation that contains equal parts of Clitoria ternatea Linn., Withania somnifera Dun., Asparagus racemosus Linn., Bacopa monniera Linn. and is used clinically as mood elevators. The aim of the present study was to explore the behavioural effects and to understand possible mode of action of Perment in stress induced depressive model. They used Chronic unpredictable mild stress (CUMS) to induce depression in rats. They used open field exploratory behaviour, elevated plus maze, social interaction and behavioural despair tests to assess behaviour. They measured plasma
noradrenaline, serotonin, corticosterone and brain/adrenal corticosterone levels using standard protocols to support the behavioural effects of Perment. They found exposure to CUMS for 21 days caused anxiety and depression in rats, as indicated by significant decrease in locomotor activity in the open field exploratory behaviour test and increased immobility period in the behavioural despair test. Further they found Perment increased the plasma noradrenaline and serotonin levels in stressed rats. No significant alteration found in the brain corticosterone level in stressed rats observed with Perment treatment. However the adrenal corticosterone level is decreased with Perment. They concluded that the Perment formulation exhibited synergistic activity, has a significant antidepressant and anxiolytic activity, which may be mediated through adrenergic and serotonergic system activation. On the basis of the results they suggested that currently the formulation is clinically used as anxiolytic but the formulation can also be indicated in patients affected with depression.

Singh et al. (2011) designed a study to find out the effects of Ashwagandha on rats and mice and found that in experimental models it increases the stamina of rats during swimming endurance test and prevented adrenal gland changes of ascorbic acid and cortisol content by swimming stress. They found pre treatment with Withania Somnifera showed significance protection against stress induced gastric ulcers. It was also found effective against urethane induced lung- adenoma in mice. It has a Cognition Promoting Effect and was useful in children with memory deficit and old age loss of memory. They suggested it is useful in neurodegenerative diseases such as Parkinson’s, Huntington’s and Alzeimer’s diseases. They suggested large scale studies are needed to prove its clinical efficicy in stress related disorders, neuronal disorders and cancer.

Konar A et al. (2011) assessed the role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain derived cells. They investigated (I) the effects of scopolamine on the molecules involved in neuronal and glial plasticity both in vivo and in vitro and
(II) their recovery by alcoholic extract of Ashwagandha leaves (i-extract). They administered scopolamine hydrobromide intraperitoneally as a drug model to mice and determined its effects on the brain function by molecular analyses. In the observations on animal system they found that the scopolamine induced cytotoxicity in IMR32 neuronal and C6 glioma cells. Furthermore, these molecules showed recovery when cells were treated with i- extract or its purified component, withanone. On the basis of their conclusions they suggested that besides cholinergic blockade, scopolamine-induced memory loss may be associated with oxidative stress and Ashwagandha i- Extract, and withanone may serve as potential preventive and therapeutic agents for neurodegenerative disorders and hence warrant further molecular analysis.

Kumar S et al. (2011) investigated the ability of an aqueous extract of W. somnifera L. Dunal (Family: Solanaceae) roots to inhibit fibril formation by the amyloid-β peptide in vitro. W. somnifera is used extensively in traditional Ayurvedic medicine as a nerve tonic with reputed memory enhancing properties. Inhibition of fibrillogenesis measured by transmission electron microscopy and ThT fluorescence assay showed that an aqueous extract of W. somnifera strongly inhibited Aβ fibril formation in a concentration-dependent manner, when compared with control samples. On the basis of the results found they suggested that the aqueous extract of W. somnifera root has an ability to inhibit the formation of mature amyloid-β fibrils in vitro, which are known to lead to amyloid plaque formation in vivo.

Soman S et al. (2012) investigated the effect of Withania Somnifera (WS) root extract and Withanolide A (WS) in restoring spatial memory deficit by inhibiting oxidative stress induced alteration in glutamnergic neurotransmission. Enhanced performance of epileptic rats treated with WS and WA was observed in Radial arm maze test. They studied the antioxidant activity of WS and WA using superoxide dismutase (SOD) and Catalase (CAT) assays in experimental rats. The SOD and CAT activity decreased significantly in epileptic group, treatment with WS and WA significantly reversed the
enzymatic activities to near control. On the basis of data they concluded that oxidative stress effects membrane constitution resulting in decreased NMDA receptor density leading to impaired spatial memory. Treatment with WS and WA has ameliorated spatial memory deficits by enhancing antioxidants system and restoring altered NMDA receptor density.

Shweta S et al. (2012) designed a study to find out the effect of *Ashwagandha* on the cardio-respiratory endurance capacity, that is, aerobic capacity of elite Indian cyclists. Forty elite (elite here refers to the participation of the athlete in at least state-level events) Indian cyclists were chosen randomly and were equally divided into experimental and placebo groups. The experimental group received 500 mg capsules of aqueous roots of *Ashwagandha* twice daily for eight weeks, whereas the placebo group received starch capsules. The baseline treadmill test for the cyclists were performed to measure their aerobic capacity in terms of maximal aerobic capacity (VO$_2$ max), metabolic equivalent, respiratory exchange ratio (RER), and total time for the athlete to reach his exhaustion stage. After eight weeks of supplementation, the treadmill test was again performed and results were obtained. There was significant improvement in the experimental group in all parameters, whereas the placebo group did not show any change with respect to their baseline parameters. There was significant improvement in the experimental group in all parameters, namely, VO$_2$ max ($t = 5.356; P < 0.001$), METS ($t = 4.483; P < 0.001$), and time for exhaustion on treadmill ($t = 4.813; P < 0.001$) in comparison to the placebo group which did not show any change with respect to their baseline parameters. They concluded Ashwagandha improved the cardio-respiratory endurance of the elite athletes.

Walvekar M et al. (2013) investigated antioxidant activity of active principles of *Withania somnifera* against D-galactose induced oxidative stress in mouse testes, epididymis and seminal vesicle. For the present investigation Swiss male albino mice Mus musculus (Linn) were used. They were grouped in to control (I), D-galactose treated (II), protective (III) and curative groups (IV).
Oxidative stress was induced in six month old mice by injecting a low dose of D-galactose. Antioxidant effect of plant extract was studied in testes, epididymis, and seminal vesicle of oxidative stressed mice on Lipid peroxidation (LPO) and fluorescence product. They found both total as well as mitochondrial lipid peroxidation and fluorescence product in testes, epididymis and seminal vesicle were increased in D-galactose induced mice. After the treatment of glycowithanolides there was significantly decrease in total as well as mitochondrial lipid peroxidation and fluorescence product in protective and curative groups. There results indicate that Withania somnifera has a capability of preventing oxidative stress and also combating stress induced infertility.

Vedi M et al. (2014) conducted a study to elucidate the protective role of Withania somnifera against bromobenzene induced nephrotoxicity and mitochondrial dysfunction in rats. Wistar albino rats of either sex were divided into six groups consisting of six animals each. The first one was control, those in group II received bromobenzene (10 mmol/kg, intragastric intubation) once, but group III and IV animals received W. somnifera (250 and 500 mg/kg, orally), respectively for 8 days followed by bromobenzene once on the 8th day and silymarin (100 mg/kg, orally) was given for 8 days to group V animals and then bromobenzene on the last day. Group VI animals received only W. somnifera (500 mg/kg) for 8 days. The levels of renal lipid peroxidation, lysosomal enzymes and glycoprotein were increased significantly (p < 0.05) in the bromobenzene alone treated rats and antioxidant status and mitochondrial enzymes were found to be decreased, when compared to the control group. The levels of kidney functional markers (urea, uric acid and creatinine) were also found to be abnormal in serum of bromobenzene alone treated rats. On the other hand, administration of W. somnifera (250 and 500 mg/kg) along with bromobenzene offered a significant dose-dependent protection to the biochemical alterations as observed in the bromobenzene alone treated rats, which was also evidenced by histopathology. Thus, the oral administration of W. somnifera significantly protected against the
Pingali U et al. (2014) Withania somnifera is an herbal medicine that has been known to possess memory-enhancing properties. They conducted a study involving an assessment of cognitive and psychomotor effects of Withania somnifera extract in healthy human participants. In this prospective, double-blind, multi-dose, placebo-controlled, crossover study, 20 healthy male participants were randomized to receive 250 mg two capsules twice daily of an encapsulated dried aqueous extract of roots and leaves of Withania somnifera or a matching placebo for a period of 14 days. Cognitive and psychomotor performance was assessed pre-dose (day 1) and at 3 hrs post-dose on day 15 using a battery of computerized psychometric tests. After a washout period of 14 days, the subjects crossed-over to receive the other treatment for a further period of 14 days as per prior randomization schedule. Significant improvements were observed in reaction times with simple reaction, choice discrimination, digit symbol substitution, digit vigilance, and card sorting tests with Withania somnifera extract compared to placebo. However, no effect can be seen with the finger tapping test. These results suggest that Withania somnifera extract can improve cognitive and psychomotor performance and may, therefore, be a valuable adjunct in the treatment of diseases associated with cognitive impairment.

Vareed SK. et al. (2014) the neuroprotective effect of Withania somnifera L. Dunal fruit extract, in rodent models, is known. Withanamides, the primary active constituents in W. somnifera fruit extract exhibited neuroprotective effects against β-amyloid-induced cytotoxicity in neuronal cell culture studies. Therefore they investigated the blood-brain barrier permeability of withanamides in W. somnifera fruit extract in mice using HPLC coupled with high resolution quadrupole time of flight mass spectrometer (Q-TOF/MS)
detection. Mice were administered with 250 mg/kg of W. somnifera extract by intraperitoneal injection, and the blood and brain samples analyzed by Q-TOF/MS detection. Four major withanamides were detected in brain and blood of mice administered with W. somnifera extract. The results suggested that the withanamides crossed the blood-brain barrier. These results may help to develop W. somnifera fruit extract as a preventive or therapeutic botanical drug for stress-induced neurological disorders.