Biological and Pharmaco-logical studies on Asgand (*Withania somnifera* Dunal) – A Review

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Abstract

sgand consists of the dried roots of *W. somnifera* (L.) Dunal. Various important medicinal values of this plant are described in the classical literature of Indian systems of medicine. This led the researchers to carry out pharmacological and biological evaluation to unravel its therapeutic potential as also to provide scientific basis of use of the plant. Results of some of the pharmacological/biological activities on the root, the most important part reputed for its medicinal value have been reviewed in this article.

Keywords: Asgand, *Withania somnifiera,*Pharmacology, Biological studies.

Introduction

Asgand is one of the promising herbal drug used in all Indian systems of medicine. Since ages Asgand being used in Uniani, Ayurveda, Siddha, Homeopathy etc. It is attributable to *Withania somnifiera* L. (Hooker, 1973) Synonyms: *Physalis somnifera*Linn., *Physallis flexuosa* Linn., *Physalis arborescens* D. C**.** In Unaniit is known as Asgand, in Ayurveda it is popularly known as Ashwagandha, in Siddha it is called Amukakara and its common english name is Winter Cherry. The plant has been historically credited to various properties like immunomodulatory, rejuvenating, anti-ageing, health promoting and reported to be used in treatment of various ailments.

Geographical Distribution: Asgand is a widespread species disseminated from the Southern Mediterranean area to the different parts of Africa and from Palestine upto North India, covering Israel, Jordan, Egypt, Sudan, Iran, Afghanistan, Sind, Morocco, Spain, Island, Srilanka and Pakistan. In India the plant grows well throughout the drier parts *viz*. Maharashtra, Gujarat, Madhya Pradesh, Rajasthan, Uttar Pradesh, Haryana, Andhra Pradesh, Karnataka and Punjab, extending to Uttranchal, Himachal Pradesh and Jammu & Kashmir from plains to a height of 1700 m. This species has been under domestication since long in Central India. Asgand is cultivated in more than 5000 hectare area in the northwestern regions of Madhya Pradesh in Mandsaur, Neemuch, Jawad and the parts of Rajasthan. (Anonymous, 1976; Atal and Schwarting, 1961; Atal *et al*., 1975; Hooker, 1973; Jaffer*et al.,* 1988; Jain *et al.,* 2007; Patra *et al.,* 2004; Purohit and Vyas, 2004).

History: The use of Asgand in Indian Systems of Medicne dates back 3000-4000 years to the teachings of the famed ayurvedic scholar Punarvasu

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Atreya. Subsequently, it was included in the writings of Charaka, Sushruta, and many other ayurvedic scholars throughout the centuries. Asgand, derived from the Sanskrit *ashva* meaning “horse” and *gandha* meaning “smell”, describes the strong aroma of the root which is considered to be reminiscent of a horse’s skin, sweat, or urine, depending upon to which authority one refers. The species name *somnifera* refers to the Latin *somnus* meaning “to sleep”, apparently alluding to the use of Asgand as a nervine and sedative. In the Unani tradition, the root was considered as a tonic, aphrodisiac and emmenagogue.Asgand is being used in the treatment (Anonymous, 2007) of Sailan-ur-Rahem (Leukaria), Jiryan (Spermatia), Riqqat-e-Mani (Attenuated Semen), Waj-ul-Qutn (Backache), Waj-ul-Mafasil (Arthraliga), Zof-e-Bah (Sexual Debility). Historically, Asgand was widely used throughout India as a tonic, especially for emaciation in people of all ages, including infants, and for enhancing reproductive function in both men and women. In one text, it was stated that Asgand taken for a fortnight with milk, ghee, oil, or warm water promotes development in an emaciated body “as rains do for younger crops”. It is classed among the “rasayanas” (rejuvenative tonics), the most highly regarded of all medicinal substances in Ayurveda. The ayurvedic scholar Charaka (100 BC) wrote of rasayanas, “One obtains longevity, regains youth, gets a sharp memory and intellect and freedom from diseases, gets a lustrous complexion, and strength of a horse”. Charaka described various uses for Asgand, including its effectiveness for treating hiccups and female disorders.Asgand historically was used for inflammation, to reduce abdominal swelling, as a mild purgative, and for the treatment of swollen glands. Asgand has been used in traditional herbal healing practices of Africa. The Southern Sotho prepared a decoction of the roots for colds and chills. The Transvaal Sotho used the root to tone the uterus in women who habitually miscarry, a use commonly employed in India as well. It has also been used to facilitate expulsion of the afterbirth. An infusion of the root bark has been used for asthma. In India it is highly regarded as a tonic and is used in formulas for a wide range of imbalances. It is cited in National formulary of Unani medicine vol. part. 1, 3, 4 and 5 (Anonymous, 2001; 2006; 2006 a; 2008), as per The Unani Pharmacopoeia of India Vol. 1 Asgand is being used in various formularies like Majoon-e-Sohag, Sonth, Majoon-e-Salab, Zimad-e-Mohallil, Kushta-e-Gaodanti(Anonymous, 2007).The Ayurvedic Pharmacopoeia of Indiain which it is cited as a strengthening tonic, aphrodisiac, and for the treatment for arthritis (Anonymous, 2001 a). It is the primary component of numerous traditional ayurvedic tonic and anti-aging compounds (Atal and Schwarting, 1961; Tripathi *et al.,* 1998; Watt and Breyer-Brandwijk, 1962).

Pharmacology

(a) Experimental Pharmacology

Antioxidant: *W. somnifiera* extract given orally for 15 days exhibited potent antioxidant defense by significantly increasing the enzymes; superoxide dismutase, catalase and ascorbic acid and showed a significant decrease in lipid peroxidation (Bhatnagar *et al*., 2005; Chaurasia *et al*., 2000). Root powder of Asgand also possessed similar kind of free radical scavenging activity (Panda *et al*., 1997) In another study, Asgand extract reduced oxidative damage in both brain regions (hippocampus and cerebral cortex) as marked by a significant decline in both lipid peroxidation and protein carbonyl in diabetic mice (Parihar *et al*., 2004). An aqueous suspension of root extract of Asgand prevented the rise of experimentally induced lipid peroxidation in rabbits and mice (Dhuley, 1998). It has decreased the activity of glutathione peroxidase (GPx) in the spinal cord from adult to aged mice and inhibited lipid peroxidation and protein oxidative modification induced by copper (Gupta *et al*., 2003). Methanolic extract of *W.somnifera* showed a dose-dependent free radical scavenging capacity and a protective effect on DNA cleavage induced by H_2O_2 UV-photholysis (Russo *et al.,* 2001). Oral treatment with Asgand root extract resulted in a significant improvement in the mice's behavior and brain antioxidant status, along with a significant reduction in the level of lipid peroxidation. Pretreatment with *W.somnifera* rootextract prevented motor impairment and significantly decreased the raised levels of malondialdehyde compared with vehicle-treated rats in the middle cerebral artery occlusion model of stroke. The protection afforded by *W. somnifera* could be due to its anti-oxidant effect (Chaudhary *et al*., 2003). Glycowithanolides of *W.somnifera* root induced a dose related increase in super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) in frontal cortex and striatum of rats (Bhattacharya *et al.,* 2001; Bhattacharya *et al.,* 2000).

Anti-tumor: *W. somnifera* exhibited anti-tumor effect in urethane-induced lung adenomas in adult male albino mice (Singh and Singh, 1986). The alcoholic extract of the dried roots of *W. somnifiera*as well as the active component withaferin A isolated from the extract showed significant anti-tumor and radiosensitizing effects in experimental tumors *in vivo*, without any noticeable systemic toxicity. Withaferin A gave a sensitizer enhancement ratio of 1.5 for *in vitro* cell killing of V79 Chinese hamster cells at a non toxic concentration of approximately 2 µM (Devi *et al.,* 1992). The growth inhibitory effect of Asgand was also observed in Sarcoma 180 (S-180), a transplantable mouse tumor. Ethanol extract of Asgand root (400 mg/kg and up, daily for 15 days) after intra-

dermal inoculation of 5 x 10^5 cells of S-180 in BALB/c mice produced complete regression of tumor after the initial growth. A 55% complete regression was obtained at 1000 mg/kg; however, it was a lethal dose in some cases. Asgand was also found to act as a radio and heat sensitizer in mouse S-180 and in Ehrlich ascites carcinoma (Devi *et al.,*1992; Devi *et al.,* 1995). Anti-tumor and radiosensitizing effects of withaferin (a steroidal lactone of WS) were also seen in mouse Ehrlich ascites carcinoma *in vivo*. *W.somnifera* hydroalcoholic root extract possessed potential chemopreventive activity on 7, 12-dimethylbenz[a] anthracene (DMBA) induced skin cancer in Swiss albino mice and the findings were supported by histopathological studies (Prakash *et al.,* 2002).The hydroalcoholic extract of the roots *W.somnifera* was screened against human laryngeal carcinoma (Hep2) cells by microculture tetrazolium assay (MTT) for anti-proliferative activity. The findings suggest that it possess cell cycle disruption and anti-angiogenic activity, which may be a critical mediator for its anti-cancer action (Mathur *et al.,* 2006). The effect of ethanolic root extract of *W. somnifiera* (REWS) against Dalton's Ascitic Lymphoma has been evaluated in Swiss albino mice. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with REWS (Christina *et al.,* 2004). *W.somnifera* (200 mg/kg, p.o.) when administered for 4 days before paclitaxel treatment and continued for 12 days caused significant reversal of neutropenia of paclitaxel in mice. The findings of the study suggest the potential of *W.somnifera* as an adjuvant during cancer chemotherapy for the prevention of bone marrow depression associated with anticancer drugs (Gupta *et al.,* 2001).Paclitaxel, administered with *W.somnifera*, may extend its chemotherapeutic effect through modulating protein-bound carbohydrate levels and marker enzymes, as they are indicators of cancer. The combination of paclitaxel with *W. somnifera* could effectively treat the benzo(a) pyrene-induced lung cancer in mice by offering protection from reactive oxygen species damage and also by suppressing cell proliferation (Senthilnathan *et al.,* 2006a). Studies on rabbits demonstrated that the tumor proteasome 5 subunit is the primary target of Withania and inhibition of the proteasomal chymotrypsin-like activity by withania *in-vivo* is responsible for, or contributes to, the antitumor effect of this ancient medicinal compound. The leaf extract kill cancer cells by at least five different pathways, viz. p53 signaling, GM-CFS signaling, death receptor signaling, apoptosis signaling and G2-M DNA damage regulation pathway (Widodo *et al.,* 2007; Widodo *et al.,* 2008). Along-term tumorigenesis study, with aniainhibited benzo (a) pyrene-induced fore stomach papillomagenesis, showing up to 60 and 92% inhibition in tumor incidence and multiplicity, respectively. Similarly, Withania inhibited 7, 12-dimethylbenzanthracene-

induced skin papillomagenesis, showing up to 45 and 71% inhibition in tumor incidence and multiplicity. The administration of Asgand rasayana (an ayurvedic polyherbal formulation containing Asgand) significantly reduced the lung tumor nodule formation by 55.6 % in experimental animal (Menon *et al.,* 1997). Simultaneous administration of withania extract and withanolide could significantly ($p < 0.001$) inhibit the metastatic colony formation of the melanoma in lungs of mice. Administration of Asgand extract was found to significantly reduce leucopenia (low white cell count) induced by cyclophosphamide treatment. Treatment of Asgand along with cyclophosphamide was found to significantly ($P < 0.001$) increase the bone marrow cellularity compared to cyclophosphamide alone treated group (Davis and Kuttan, 1998). Administration of an extract from the powdered root of the plant Asgand enhanced the levels of Interferon gamma (IFN-gamma), Interleukin-2 (IL-2) and Granulocyte macrophage colony stimulating factor (GM-CSF) in normal Balb/c mice (Davis and Kuttan, 1999). Treatment with Asgand had normalised the ratio of normochromatic erythrocytes and polychromatic erythrocytes in mice after the radiation exposure. Major activity of Asgand seemed to be in the stimulation of stem cell proliferation. Administration of Asgand (20 mg/dose/animal) for five days in conjunction with cyclophosphamide was associated with a reduction in urotoxicity. In a further study at the same dose it was found to inhibit the 20-methylcholanthrene induced sarcoma development in mice and increased the survival rate to 100% of tumor bearing animals (Davis and Kuttan 2000a; Davis and Kuttan, 2002a; Davis and Kuttan, 2002b).Oral administration of *W.somnifera* root extract reduced the tumor incidence, tumor volume and enhanced the survival of the mice compared with 20-methylcholanthrene injected mice (Prakash *et al.,* 2002).Pretreatment of rats with 1-oxo-5[beta], 6[beta]-epoxy-witha-2-enolide (20 mg/kg bwt.) isolated from the roots of *W. somnifera*, prevents the incidence of UV B radiation induced skin carcinoma and also prevents malignancy in the cutaneous tissue (Mathur *et al.,* 2006). An in vitro study showed withanolides from *W. somnifiera* inhibited growth in human breast, central nervous system, lung and colon cancer cell lines comparable to doxorubicin. Withaferin A more effectively inhibited growth of breast and colon cancer cell lines than did doxorubicin. These results suggest it may prevent or inhibit tumor growth in cancer patients and suggest a potential for development of new chemotherapeutic agents (Jayaprakasam *et al.,* 2003). Further withanolides inhibit the activation of NF-kappa B and NFkappaB-regulated gene expression, which may explain the ability of withanolides to enhance apoptosis and inhibit invasion and osteoclastogenesis (Ichikawa *et al.,* 2006). Treatment with *W. somnifiera* inhibited ochratoxin A (OTA) induced suppression of chemotactic activity and

production of interleukin-1 (IL-1) and tumour necrosis factor by macrophases (Dhuley, 1998).

Antibacterial: Both aqueous as well as alcoholic extract of *W. somnifiera* (roots as well as leaves) were found to possess strong antibacterial activity against a range of bacteria, as revealed by *in vitro* Agar Well Diffusion Method. Moreover, in contrast to the synthetic antibiotic (viz. chloramphenicol), the extracts did not induce lysis on incubation with human erythrocytes, advocating their safety to the living cells (Owais *et al.,* 2005). Methanol and hexane extract of both leaves and roots of *W. somnifiera* (WS) exhibited potent antibacterial activity against *Salmonella typhimurium* and *Escherichia coli* by agar plate disc-diffusion assay. A synergistic increase in the antibacterial effect of Tibrim (combination of rifampicin and isoniazid) was noticed when MIC of Tibrim was supplemented with the WS extract (Arora *et al.,* 2004).

Spermatogenic: Lyophilized aqueous extract of *W. somnifiera* produced increase testicular weight and found to have a direct spermatogenic influence on the seminiferous tubules of immature rats presumably by exerting a testosterone-like effect (Abdel-Magied *et al.,* 2001).

Cardiovascular: *W. somnifiera* (50 mg/kg) exerts a strong cardioprotective effect in the experimental model of isoprenaline-induced myonecrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardioprotective effect. *W.somnifera* had a prolonged hypotensive, bradycardia, and respiratory-stimulant action in dogs (Malhotra *et al.,* 1965). Significant increase in relative heart weight and glycogen content in heart and liver was observed in the extract treated animals. It increased the duration of contractility of frog heart muscle and resulted in significant increase in coagulation time which attains normalcy seven days after cessation of treatment. The ethanolic and aqueous extract exhibited low angiotensin-converting enzyme (ACE) inhibitory activity (Nyman *et al.,* 1998).

Sexual Behaviour: Methanolic root extract of *W. somnifera* induced a marked impairment in libido, sexual performance, sexual vigour, and penile erectile dysfunction. These effects were partly reversible on cessation of treatment. This antimasculine effect was not due to changes in testosterone levels but attributed to hyperprolactinemic, GABAergic, serotonergic or sedative activities of the extract. *W. somnifera* roots may be detrimental to male sexual competence (Ilayperuma *et al.,* 2002).

Anti-Ulcer: *W. somnifiera* root extract given orally (100 mg/kg BW/day p.o.) for 15 days significantly reduced the ulcer index, volume of gastric secretion, free acidity and total acidity in indomethacin and restraint induced gastric ulcer rats (Bhatnagar *et al.,* 2005). Asgand kwatha (equivalent to 1000 mg/kg) exhibited protective effect against rifampicin and isoniazid induced liver damage in mice (Chhajed *et al.,* 1991). The root powder produced dose dependent significant antiulcerogenic effect on aspirin induced gastric ulcers in rats.The equimolor combination of sitoindoside VII, sitoindoside VIII and withaferin A and hydromethanolic extractives of the roots reduced the incidence and the severity of restraint stress induced gastric ulcers in rats (Bhattacharya *et al.,* 2000).

Hypolipidemic: When the root powder of *W. somnifiera* was added to the diet at 0.75 and 1.5 gm/rat/day, hypercholesteremic animals registered significant decreases in total lipids, cholesterol and triglycerides in plasma. On the other hand, significant increases in plasma HDL-cholesterol levels, HMG-CoA reductase activity and bile acid content of liver were noted in these animals. A similar trend was also noted in bile acid, cholesterol and neutral sterol excretion in the hypercholesteremic animals with *W. somnifera* administration. Further, a significant decrease in lipid-peroxidation occurred in *W.somnifera* administered hypercholesteremic animals when compared to their normal counterparts. However, it appeared that *W. somnifera* root powder is also effective in normal subjects for decreasing lipid profiles (Visavadiya *et al.,* 2007). The root powder given for 30 days in a dose of 3 g/day in a small clinical trial of six NIDDM and six hyper-cholesterolemic patients exhibited hypoglycaemic, diuretic and hypolipidaemic effects.

Hepatoprotective: Iron overload induced increase in hepatic lipid peroxidation and serum levels of the enzymes, were attenuated by *W. somnifera* glycowithanolides in a dose related manner which explains their hepatoprotective action against heavy metals and other environmental toxins in rats (Bhattacharya *et al.,* 2000). Withaferin a reduced CCl_4 induced hepatotoxicity in rats (Sudhir and Budhiraja, 1992). The liver and kidney of rat underwent severe histopathological lesions when treated with a single bolus dose of carbendazim, a fungicide, particularly affecting the hepatocytes and the renal corpuscles, respectively. The effects appear to be manifestations of the microtubule-disrupting activity of carbendazim. Treatment of carbendazim-treated rats with the powder of tuberous root of *W. somnifiera* for 48 days resulted in complete cure of these organs (Akbarsha *et al*., 2000).

Thyroid Stimulating: Daily administration of *W. somnifiera* root extract (1.4g/kg body wt) for 20 days on thyroid function in female mice showed

an increase in hepatic glucose-6-phosphatase(G-6-Pase) activity and antiperoxidative effects as indicated either by a decrease in hepatic lipid peroxidation (LPO) and by an increase in the activity of antioxidant enzymes. The results indicates its thyroid stimulating function. These results indicate Asgand may be a useful botanical in treating hypothyroidism (Panda *et al*., 1997; Pandaand Kar, 1997).

Anti-Inflammatory: *W. somnifiera* root extract (1g/kg, oral) reduced Freund's complete adjuvant induced inflammation in rats; phenylbutazone was given as a positive control. The α2- glycoprotein (an indicator useful for diagnostic and prognostic assessment of arthritic and inflammatory conditions) found only in inflamed rat serum was decreased to undetectable levels in the *W.somnifera* group. Phenylbutazone, on the other hand, caused a considerable increase in the α2-glycoprotein in both arthritic and healthy rats (Anabalagan and Sadique, 1985; Anabalaganand Sadiques, 1981).*W. somnifiera* root powder also decreased air pouch granuloma induced by carrageenan on the dorsum of rats. *W. somnifera* decreased the glycosaminoglycans content in the granuloma tissue more than hydrocortisone treatment. Italso uncoupled the oxidative phosphorylation by significantly reducing the ADP/O ratio in mitochondria of granuloma tissue (Begum and Sadique, 1987). In a different study, *W. somnifera* root extract (1000mg/ kg, oraly daily for 15 days) caused significant reduction in both paw swelling and bony degenerative changes in Freund's adjuvant-induced arthritis in rats as observed by radiological examination. The reductions were better than those produced by the reference drug, hydrocortisone (Begum and Sadique, 1988). It has inhibited the granuloma formation in cotton-pellet implantation in rats and the effect was comparable to hydrocortisone sodium succinate (5 mg/kg) treatment. In another study 80% ethanolic extract of *W.somnifera* possessed anti-inflammatory activity in carrageenan-inducedrat paw edema model.Few studies have been conducted on the mechanism of action for the anti-inflammatory properties of *W. somnifera.* In one study, rats injected with formaline in the hind leg footpad showed a decrease in absorption of ^{14}C-glucose in rat jejunum (Somasundaram *et al.,* 1983). Glucose absorption was maintained at the normal level by both *W. somnifera* and the cyclooxygenase inhibitor oxyphenbutazone. Both drugs produced anti-inflammatory effects. Similar results were obtained in parallel experiments using ^{14}C-leucine absorption from the jejunum (Somasundaram *et al.,* 1983). These studies suggest cyclooxygenase inhibition may be involved in the mechanism of action of *W. somnifera*.

Anti-Stress: Hydroalcoholic extract of *W. somnifiera* roots (50 mg/kg b.w orally once daily for 21 days) containing glycowithanolides [WSG] normalized chronic foot-shock stress induced increase in superoxide dismutase (SOD) and reversed the decrease in catalase (CAT) and glutathione peroxidase (GPX) values in both the brain areas (frontal cortex and striatum) of rats, indicating its anti-stress adaptogenic property (Bhattacharya *et al.,* 2001). In another study, root extract and equimolar combination of Sitoindosides VII, VIII and Withaferin-A were uniformly effective in attenuating restraint stress induced responses ranging from anxiety, depression, analgesia, thermic changes, gastric ulcers, convulsions, tribulin activity and adrenocortical activation in rats (Bhattacharya *et al.,* 1997). It reversed the cold swimming-induced increases in plasma corticosterone, phagocytic index and avidity index to control levels. *W. somnifera* root powder (100 mg/kg orally as an aqueous suspension daily for seven days) given before the swimming test in water at 10ºC also increased total swimming time, indicating better stress tolerance in rats (Archana and Namasivayam, 1999; Dhuley, 2000). The aqueous suspension of root extract of Asgand prevented the rise in lipid peroxidation in stress induced rabbits and mice (Dhuley, 1998). A new withanolide, 1-oxo-5 beta, 6 beta-epoxy-witha-2-ene-27-ethoxy-olide from aqueous extract of *W. somnifiera* exhibited adaptogenic activity on stress indices in the cold-hypoxia-restraint (C-H-R) model (Kaur *et al.,* 2003). The extract of Asgand (1:1 aqueous–methanolic extract; doses of 20, 50, 100 mg/kg ip.) and/or sitoindoside administered to mice and rats improved memory related performance in passive avoidance tasks and protected against stress induced response, ranging from anxiety, depression, thermic changes, gastric ulcers, convulsions and tribulin activity. This was accompanied by a preservation of adrenal ascorbic acid and corticosterone levels suggesting that a corticosterone sparing effect is one of the mechanisms of action of adaptogens (Singh *et al.,* 1982). Experimental rats were subjected to immobilization stress for 14 h and were treated with a root powder extract of *W. somnifera.* Control rats were maintained in completely, non stressed conditions. Thionin stained serial coronal sections (7 microm) of brain passing through the hippocampal region of stressed rats (E(1) group) demonstrated 85% degenerating cells (dark cells and pyknotic cells) in the CA(2) and CA(3) sub-areas. Treatment with *W.somnifera* rootpowder extract significantly reduced (80%) the number of degenerating cells in both the areas. The study thus demonstrates the antistress neuroprotective effects of *W. somnifera* (Jain *et al.,* 2001). Rat model of chronic stress (CS) induced significant hyperglycaemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression were attenuated by

W. somnifera (25 and 50 mg/kg po) administered 1 h before footshock for 21 days. The results indicate that *W. somnifera* has significant antistress adaptogenic activity, confirming the clinical use of the plant in Ayurveda (Bhattacharya *et al.,* 2002). In another study a new withanolide free hydro soluble fraction from roots of *W. somnifera* exhibited significant antistress activity in a dose dependent manner (Singh *et al.,* 2003; Singh *et al.,* 2001). An herbal formulation comprising of Asgand showed adaptogenic activity comparable to *Panax ginseng* against a variety of behavioral, biochemical and physiological perturbations induced by unpredictable stress in CF strain albino rats (Bhattacharya *et al.,* 2000). Sitoindosides VII and VIII from Asgand significantly suppressed immobilization stress induced increase in corpus striatum dopamine receptors in rats (Saksena, 1989).

Nootropic: Oral administration of Asgand attenuated the disruption of memory consolidation produced by chronic treatment with electroconvulsive shock and reversed the scopolamine induced delay in transfer latency in mice. On the basis of these findings, it has been suggested that Asgand exhibits a nootropic-like effect in naive and amnesic mice (Dhuley, 2001). Methanolic extract of Asgand showed potent inhibition of AChE activity, indicating its role in improvement of cognition (Vinutha *et al.,* 2007). Asgand extract showed cognition enhancing and memory-improving effects by increasing cortical muscarinic acetylcholine receptor activity (Schliebs *et al.,* 1997).Withanolide derivatives isolated from methanol extract of Asgand, showed neurite extension in normal and damaged cortical neurons and also showed neurite outgrowth in human neuroblastoma SH-SY5Y cells (Zhao *et al.,* 2002). In normal cortical neurons, the predominant dendritic out-growth was induced by treatment with withanoside IV or withanoside VI, whereas predominant axonal outgrowth was observed in treatment with withanolide A (Kuboyama *et al.,* 2005). Aß(25-35) or Amyloid beta is a major pathological cause of Alzheimers disease due to formation of a beta sheet structure and induces neuroal cell death, neurite atrophy, synaptic loss and memory impairment. Simultaneous treatment with Aß(25-35) and withanolides from Asgand significantly prevented both dendritic and axonal atrophy induced by Aß(25-35) in rat cortical neurons (Dadkar *et al.,* 1987). Extension of dendrites and axons in neurons may compensate for and repair damaged neuronal circuits in the dementia brain. Methanol extract of Asgand (roots of *W. somnifera*; 5 microg/ml) significantly increased the percentage of cells with neurites in human neuroblastoma SK-N-SH cells. These results suggest that the methanol extract of Asgand promotes the formation of dendrites (Kuboyama *et al.,*2002; Tohda *et al.,* 2000).

Nervous System: *W. somnifiera* significantly inhibited haloperidol or reserpine-induced catalepsy and provide hope for treatment of Parkinson's disease. 6-Hydroxydopamine (6-OHDA) is one of the most widely used rat models for Parkinson's disease. There is ample evidence in the literature that 6-OHDA elicits its toxic manifestations through oxidant stress. *W. somnifiera* reversed all the toxic manifestations induced by 6-OHDA in a dose dependent manner (Ahmad *et al.,* 2005). *W. somnifiera* glycowithanolides (WSG) administered concomitantly with haloperidol for 28 days, inhibited the induction of the neuroleptic tardive dyskinesia. Antioxidant effect of WSG, rather than its GABA-mimetic action reported for the prevention of haloperidol-induced tardive dyskinesia (Bhattacharya *et al.,* 2002). Chronic Asgand treatment was effective in preventing the behavioral deficit in depressive animal models, which was accompanied by an adaptive supersensitivity of postsynaptic 5HT2 receptors (Tripathi *et al.,* 1998).It also has got a significant anticonvulsive property (Rai *et al.,* 1983). Glycowithanolides (WSG) isolated from *W. somnifiera* roots exhibited potent anxiolytic and antidepressant actions in rats. The activity was comparable to those elicited by the benzodiazepine, lorazepam for anxiolytic studies and by the tricyclic anti-depressant, imipramine for the antidepressant investigations (Bhattacharya *et al.,* 2000).

Anti-Venom: Venom hyaluronidases help in rapid spreading of the toxins by destroying the integrity of the extra-cellular matrix of the tissues in the victims. A hyaluronidase inhibitor (WSG) is purified from *W. somnifera*. The glycoprotein inhibited the hyaluronidase activity of cobra (*Naja naja*) and viper (*Daboia russelii*) venoms, which was demonstrated by zymogram assay and staining of the skin tissues for differential activity. WSG completely inhibited the activity of the enzyme at a concentration of 1:1 w/w of venom to WSG. External application of the plant extract as an antidote in rural parts of India to snakebite victims appears to have a scientific basis (Machiah *et al.,* 2006). In another study antitoxin-PLA2 glycoprotein isolated from *W.somnifera* neutralized the PLA2 activity of the *Naja naja* venom (Lizano *et al.,* 2003).

Immunomodulatory: In a mouse study, *W. somnifiera* root extract enhanced total white blood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group (Davis and Kuttan, 2000a; Davis and Kuttan, 2002a). *W. somnifiera* extract indirectly modulates immune activity and probably disengages *Listeria monocytogenes* induced suppression of immune responses by inducing a higher reserve of myeloid progenitors in the bone marrow, proliferation of lymphocytes and increased INF-γ levels (Teixeira *et al*., 2006). Methanolic extract of *W. somnifera* root increased inducible nitric

oxide synthase protein expression in a concentration dependent fashion in mouse macrophages. The increased NO production by macrophages could account for the immunostimulant properties of *W. somnifera* (Iuvone*et al.,* 2003). In different study with the aqueous suspension of *W. somnifiera* root powder was investigated for their *in vivo* and *in vitro* immunomodulatory properties. *W. somnifera* showed potent inhibitory activity towards the complement system, mitogen induced lymphocyte proliferation and delayed-type hypersensitivity reaction. Administration of *W.somnifera* root powder did not have a significant effect on humoral immune response in rats (Rasool and Varalakshmi, 2006). Glycowithanolides and a mixture of sitoindosides IX and X isolated from *W.somnifera*, both produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes. Root extract of *W. somnifera* was tested for immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprine and prednisolone (Ziauddin *et al.,* 1996). *W. somnifiera* root extract was found to stimulate immunological activity in Babl/c mice. Treatment with Withania root extract (20 mg/dose/animal; i.p.) was found to increased significantly ($P<0.001$) the total WBC count, bone marrow cellularity as well as alpha-esterase positive cell number. When treated along with the antigen (SRBC) produced an enhancement in the circulating antibody titre and the number of plaque forming cells (PFC) in the spleen. It also inhibited delayed type hypersentivity reaction in mice (Mantoux test) and showed an enhancement in phagocytic activity of peritoneal macrophages when compared to control in mice (Davis and Kuttan, 2000). Treatment of immunized animals (DBT) with standardized aqueous extract of *W. somnifiera* (100 mg/kg/day) for 15 days resulted in significant increase of antibody titers to B. pertussis (Gautam *et al.,* 2004).On oral administration, Asgand churna showed a significant increase in neutrophil adhesion and delayed-type hypersensitivity (DTH) response. It is concluded that Asgand churna significantly potentiated the cellular immunity by facilitating the footpad thickness response to SRBCs in sensitized rats.Pretreatment with a polyherbal formulation contianing *W. somnifiera* extract increased proliferation of splenic leukocyte to B cell mitogen, lipopolysaccharidic and cytotoxic activity against K 562 cells in mice (Nemmani*et al.,* 2002). Withaferin A and withanolide E, two sterioudal lactones of withania were demonstrated to have specific immunosuppresive effects on human B and T lymphocytes as well as on mice thymocytes (Shohat*et al.,* 1978). *W. somnifera* given orally once daily for 7 consecutive days in a dose of 100 mg/kg after intravenous infection of *Aspergillus fumigatus* prolonged the survival period of infected mice. This protective activity was probably related to the observed increases

in phagocytosis and intracellular killing of peritoneal macrophages induced by Ashwaganda treatment (Tohda *et al.,* 2000).Cyclophosphamide-induced immunosuppression was counteracted by treatment with *W. somnifera* extract, revealing significant increase in hemagglutinating antibody responses and hemolytic antibody responses towards sheep red blood cells (Gautam*et al.,* 2004; Agarwal *et al.,* 1999). The effects of graded doses of a chemically standardized aqueous alcoholic (1:1) root extract (AGB) of *W. somnifera* on the immune system of SRBC immunized BALB/c mice were investigated. Mice were administrated AGB orally for 15 days. AGB stimulated cell mediated immunity, IgM and IgG titers reaching peak value with 30 mg/kg b.wt. Flow cytometric analysis of lymphocyte surface markers of T cells ($CD3^+$, $CD4^+$ and $CD8^+$) and B cells ($CD19^+$) indicated prominent enhancement in proliferation and differentiation of lymphocytes. The extract selectively, induced type 1 immunity because it guided enhanced expression of T helper cells (Th)1 cytokines interferon (IFN)-γ and interleukin (IL)-2 while Th2 cytokine IL-4 observed a moderate decline. Confirmation of Th1 polarization was obtained from augmented levels of IgG2a over IgG1 in the blood sera of AGB treated groups. Withanolide-A, a major constituent of AGB appeared responsible for Th1 skewing effect of the extract as it significantly increased the levels of Th1 cytokines, decreased moderately IL-4 and significantly restored the selective dexamethasone inhibition of Th1 cytokines in mouse splenocytes cultures *in vitro*. In addition, AGB also strongly activated macrophage functions in*vivo* and *in vitro* indicated by enhanced secretion of nitrite, IL-12 and TNF-α. In contrast IL-10 remained unchanged again suggesting that AGB critically influenced Th1 profile of the cytokines. The studies suggested that AGB supports predominantly Th1 immunity with increase in macrophage function. Glycowithanolides and mixture of sitoindosides IX and X isolated from *W. somnifiera* produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes indicating its immunomodulatory potential (Ghosal *et al.,* 1989).

Antiviral: Hydroalcoholic extract showed poor *in vitro* anti-HIV activity. Methanolic extract did not show any *in vitro* and *in vivo* inhibitory effect on *Herpes simplex virus* (Hattori *et al.,* 1995).

Endocrinology: Ethanolic extract prevented *in vitro* glucose-mediated collagen glycation and cross-linking which is the process involved in end-organ damages in diabetes mellitus. The activity of the ethanolic extract was comparable to metformin, an antiglyeating agent and it showed more prominent effect than the root powder in rats (Babu *et al.,* 2007).

Ocular/Ophthalmic Effects: Withaferin A and withanolide D exhibited potential effect against choroidal neovascularization in eye (Bargagna *et al.,* 2006).

Antirheumatic: In a non-randomized trial in 118 patients, the root powder was found to provide relief in cases of acute rheumatoid arthritis and acute exacerbation of chronic arthritis.It also showed marked improvement in 22% of the 77 patients cases and moderate response is 53 % case of rheumatoid arthritis in a single blind clinical trial. Patients suffering from chronicity of diseases less than a year and 1-2 years and mild to moderately severe diseases showed better results (Bikshapathi and Kumari, 1999).

Antifungal: Withaferin A exhibited antifungal activity against *Aspergillus niger, Candida albicans* and *T. rubrum* (Dasgupta *et al.,* 1970).

Radiosensitivity: Withaferin A modified the effect of radiotherapy on bone marrow cell survival of the mouse, as studied using exogenous spleen colony unit (CFU-S) assay and the effect was compared with cyclophasphamide (CP). Withaferin A alone produced the lower number of CFU-S as compared with normal control but had a lower cytotoxicity compared with CP. But in combination with radiotherapy, the Withaferin A modified the effect of radiotherapy, significantly enhancing the cell lethality to the same extent as combination of CP + RT (Ganasoundari *et al.,* 1997).

Further Reported Activities

Ethanolic extract of *W. somnifiera* displayed cytotoxic effects on FL-cells using the neutral red assay (Ali *et al.,* 2001) and exhibited potent anti-osteoporotic activity in female rats (Nagareddy and Lakshmana, 2006). It also exhibited most significant in vitro enzyme inhibition activities against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase enzymes (LO) (Khattak *et al.,* 2005). *W. somnifiera* exhibits cytoprotective property (Shukla *et al.,* 2000). A polyherbal formulation containing aqueous extract of *W. somnifiera* proved to have hypoglycemic activity in normal and streptozotocin induced diabetic mice.

(b) Clinical Pharmacology

Anxiolytic: In a study involving a total of 39 patients (20 receiving the drug and 19 received placebo) in a double-blind placebo-controlled trial in patients of anxiety disorders the ethanolic extract was found to exert significant anxiolytic effects (Andrade *et al.,* 2000).

Hypolipidemic: When six mild hypercholesterolemic subjects were treated with the powder of roots of *W.somnifera* for 30 days significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol were observed indicating that root of *W. somnifera* is a potential source of hypocholesterolemic agent (Andallu and Radhika, 2000).

Anti-inflammatory: One clinical trial supports the possible use of *W.somnifera* for arthritis. In a double-blind, placebo-controlled cross-over study, 42 patients with osteoarthritis was randomized to receive a formula containing Asgand or placebo for three months. Patients were evaluated for one month pretreatment, during which time all previous drugs were withdrawn. During both the pretreatment and treatment phase, pain and disability scores were evaluated weekly while erythrocyte sedimentation (SED) rate and radiological studies were conducted monthly. The herbal formula significantly reduced the severity of pain ($p<0.001$) and disability ($p<0.05$) scores, although no significant changes in radiological appearance or SED rate were noted (Kulkarni*et al.,* 1991).

Anti-Aging: In a double-blind clinical trial, Asgand was tested in a group of 101 healthy males, 50-59 years old, at a dosage of 3 grams daily for one year. A significant improvement in hemoglobin, red blood cell count, hair melanin, and seated stature was observed. Serum cholesterol decreased and nail calcium was reserved. Erythrocyte sedimentation rate decreased significantly and 71.4 % reported improvement in sexual performance (Kuppurajan *et al.,* 1980). In a double-blind clinical trial on children (8-12 years of both sex), Asgand increased body weight, total proteins and mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration significantly. The combination of punarnava and Asgand increased significantly haemoglobin and mean corpuscular haemoglobin concentration levels. This study indicated Asgand usefulness as haematinic and growth promoters in the growing children (Venkataraghavan*et al.,* 1980).

Anti-Diabetic: The hypoglycemic, diuretic and hypocholesterolemic effects of roots of *W. somnifera* were assessed in six mild NIDDM subjects and six mild hypercholesterolemic subjects. The treatment consisted of the powder of roots over a 30 day period. At the end of the study, researchers noted a decrease in blood glucose comparable to that of oral hypoglycemic drug and a significant increase in urine sodium, urine volume, significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol, with no adverse effects (Andallu and Radhika, 2000).

Nootropic: In a placebo-controlled study, men and women given an extract of Asgand root showed improved mental skills by performing better in reaction times, mental arithmetic and logical deductions (Karnick, 1991). In another placebo-controlled double blind study, Ayurvedic capsules containing one of the ingredients as Asgand, appears to be superior in improving psychomotor function compared to placebo (Karnick, 1992). The methanolic extract exhibited potent acetylcholinesterase inhibitory activity *in vitro* and it was found more active than the aqueous extract which suggests its potential in Alzheimer disease (Vinutha *et al.,* 2007).

Immunomodulatory: The polyherbal drug Immu-25 showed a favourable effect in patients (36: 10 female and 26 male) with HIV infection. The test drug decreased the mean viral load, which was associated with good symptomatic improvement and an increase in the mean CD4 cell count. On the basis of these data, it can be concluded that this herbal drug may have a good immunomodulatory effect and has potential as a co-therapeutic agent in the management of HIV infection (Usha *et al.,* 2003)**.**

Conclusion

From the above it is evident that Asgand is one of the most important herbal drugs having scientically proven therapeutic efficacy.

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