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REVIEW

Pharmacologic overview of *Withania somnifera*, the Indian Ginseng

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Abstract *Withania somnifera*, also called 'Indian ginseng', is an important medicinal plant of the Indian subcontinent. It is widely used, singly or in combination, with other herbs against many ailments in Indian Systems of Medicine since time immemorial. *Withania somnifera* contains a spectrum of diverse phytochemicals enabling it to have a broad range of biological implications. In preclinical studies, it has shown anti-microbial, anti-inflammatory, anti-tumor, anti-stress, neuroprotective, cardioprotective, and anti-diabetic properties. Additionally, it has demonstrated the ability to reduce reactive oxygen species, modulate mitochondrial function, regulate apoptosis, and reduce inflammation and enhance endothelial function. In view of these pharmacologic properties, *W. somnifera* is a potential drug candidate to treat various clinical conditions, particularly related to the nervous system. In this review, we summarize the pharmacologic characteristics and discuss the mechanisms of action and potential therapeutic applications of the plant and its active constituents.

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Keywords *Withania somnifera* · Anti-bacterial · Anti-inflammatory · Anti-arthritic · Anti-cancer · Cardio-protective · Anti-diabetic · Anti-stress · Parkinson's disease · Alzheimer's disease · Stroke hypoxia

Abbreviations

TNF-α	Tumor necrosis factor-α
IL-1β	Interleukin-1β
NFκ-β	Nuclear factor kappa-β
NO	Nitric oxide
ROS	Reactive oxygen species
PARP-1	Poly(ADP-ribose) polymerase-1
pMCAO	Permanent middle cerebral artery occlusion
GFAP	Glial fibrillary acidic protein
6OHDA	6-hydroxydopamine

Introduction

Withania somnifera (*W. Somnifera*) is a small woody shrub commonly known as "Winter cherry" or "Indian Ginseng". In Sanskrit it is known as 'Ashwagandha' and in Urdu as 'Asgand' [1, 2]. It belongs to the family Solanaceae and attains a height of 0.5–2 m. The plant is widely distributed in the drier parts of tropical and subtropical zones ranging from the Canary Islands, South Africa, Middle East, Sri Lanka, India and to China. It is cultivated in gardens in the warmer parts of Europe and has become a wild weed in some parts of Australia [3, 4]. However, in India it is grown as a medicinal crop [5]. The whole plant or its different parts are widely used in Ayurvedic and Unani systems of medicine (indigenous systems of medicine in India) for its medicinal properties and has been used since

antiquity. The plant is mentioned as an official drug in Indian Pharmacopoeia-1985 [6, 7].

In Ayurveda it is a prominent herbal Rasayana and is known as "Sattvic Kapha Rasayana". Rasayana is a herbal or metallic preparation that is used for pharmacologic properties such as/in adaptogenic, aphrodisiac, tonic, narcotic, diuretic, anti-helminthic, astringent, thermogenic and stimulant, anti-stress, anti-inflammation, anti-carbuncle, anti-ulcer, debility from old age, rheumatism, vitiated conditions of vata, leucoderma, constipation, insomnia, nervous breakdown, goiter, leucorrhoea, boils, pimples, flatulent colic, worms, piles, and oligospermia [8–13]. Additionally, it is prescribed for snake venom and scorpion sting [14–16]. In the Unani system of medicine, the plant has been mentioned in an old testament "Kitab-ul-Hashaish" by Dioscorides in 78 AD. In Unani, Asgand has various therapeutic uses. Asgand has been recommended for the treatment of various ailments, which include arthritis, lumbago, carbuncle, spermatorrhoea, asthma, leucoderma, general debility, sexual debility, anxiety, neurosis, scabies, ulcers, and leucorrhoea [6, 17–21]. Owing to its pronounced stress-busting qualities, the plant has been given its species name 'somnifera', which is a Latin word meaning 'sleep-inducer' [22, 23]. Pharmacologic effects and folkloric uses of *W. somnifera* are akin to that of Korean Ginseng tea, which furnishes a modest explanation for calling *W. somnifera* as Indian Ginseng [24].

In Unani and Ayurvedic systems of medicine, mostly roots of *W. somnifera* are used for the therapeutic purposes. The plant loses it pharmacologic activity after 2 years, therefore freshly dried roots are preferred for good results [6, 7]. The leaves of the plant are bitter and have some medicinal uses in fever and painful swelling. The flowers are astringent, depurative, diuretic, and aphrodisiac. The seeds are anti-helminthic, remove white spots from the cornea, increase sperm count, as well as testicular growth. The fruits have been traditionally used as a topical treatment for tumors and tubercular glands, carbuncles, and skin ulcers [7, 25, 26].

Chemical composition

Phytochemical studies have shown the presence of different chemical constituents in various regions of *W. somnifera.* More than 12 alkaloids, 40 withanolides and several sitoindosides have been isolated and reported from the plant [27]. The major chemical constituents of *W. somnifera* are (Table 1):

Alkaloids	Withanine, withananine, withasomnine, somniferine, tropeltigloate, somniferinine, somninine and nicotine [28]
Steroidal lactones	Withaferin-A, withanone, withanolide-E, withanolide-F, withanolide-A, withanolide-G, withanolide-H, withanolide-I, withanolide-J, withanolide-K, withanolide-L, withanolide-M [27, 28]
Steroids	Cholesterol, β-sitosterol, stigmasterol, diosgenin, stigmastadien, sitoinosides VII, sitoinosides VIII, sitoinosides IX, sitoinosides X [27, 29]
Salts	Cuscohygrine, anahygrine, tropine, pseudotropine, anaferine [30]
Flavonoids	Kaempferol, quercetin [29]
Nitrogen-containing compounds	Withanol, somnisol, and somnitol [28]

Table 1 Bio-active compounds in the different parts of the plant

Part of plant	Bio-active compounds present
Root	Vitoindosides VII, VIII (acylsteryl-glucoside) [31], sitoindosides IX, X (glycowithanolide) [32], withanine, withananine (alkaloids), withanolide-A, viscosa lactone-B, stigmasterol, stigmasterol [33–35] and ashwagandhanolide [27, 36]
Leaf	Withaferin [37], withaferin-A, withanone, withanolide-D, withanolide-E, withanolide-B, 27-deoxywithaferin-A, 2, 24-dienolide, trienolide (steroidal lactones), withanoside-IV, withanolide-Z, 7-hydroxywithanolide, 3α-methoxy-2, 3-dihydro, 4β, 17α-dihydroxy-1-1oxo,5β, 6β-epoxy-22R-witha, 4β-dihydroxy-5β, 6β-epoxy, 1-oxo-22R-witha-2, 14–24 [38–44]
	Sitoindoside IX, 4-(1-hydroxy-2, 2-dimethylcyclo propanone, 2, 3-dihydrowithaferin-A, 2, 3-dihydrowithaferin-A, 24, 25-dihydro-27 desoxywithaferin-A, physagulin-D, physagulin-D (1–>6)-β-D-glucopyranosyl- (1–>4)-β-D-glucopyranoside, 27-*O*-β-D-glucopyranosylphysagulin-D, 27-*O*-β-D- lucopyranosylviscosalactone-B, 4, 16-dihydroxy-5β, 6β-epoxyphysagulin-D, viscosalactone–B [45, 46]
	5, 20α (R)-dihydroxy-6α, 7α-epoxy-1-oxo- (5α) -witha-2, 24-dienolide (steroidal lactone)2, 3-dihydrowithaferin-A-3β-*O*-sulfate [47]
Fruit	5β, 6α, 14α, 17β, 20β-pentahydroxy-1-oxo-20S, 22R-witha-2,24-dienolide, 6α,7α-epoxy-5α,14α,17α,23β- tetrahydroxy-1-oxo-22R-witha-2,24-dienolide, 7α-hydroxy withanolide, withanolide glycosides, 17α- and 17β-withanolides, Withanone, 27-hydroxy withanolide- A [48–50]
Seed	Withanolide-WS-2 (aliphatic ester), withanolide-WS-1 (aliphatic ketone) [49, 51, 52]

Toxicologic studies

Withania somnifera has been used for various pharmacological activities for very long time for all age groups and both sexes and even during pregnancy without toxic effects [13]. Prabu et al. [53] have evaluated hydro-alcoholic root extract of *W. somnifera* against acute and sub-acute oral toxicities in Wistar rats and found it non-toxic even at 2000 mg/kg body weight. The extract was administered at 2000 mg/kg and observed for 14 days for acute toxicity and at 500, 1000 and 2000 mg/kg and observed for 28 days for sub-acute toxicity, however there was no significant change in body weight, organ weight, and hemato-biochemical parameters. In addition, the toxicity profile of *W. somnifera* was assessed on the developing fetus of pregnant rats including mortality, structural abnormalities, and changes in growth but no evident changes were found in the mother or in the fetus. No changes were found in the body weight of prenatal females, number of corpora lutea, implantations, viable fetuses, and skeletal and visceral formations [54]. Acute and sub-acute toxicity studies in Swiss albino mice and Wistar rats administrated with intraperitoneal injections of 1100 mg/kg did not produce any deaths within 24 h but small increases have led to mortality with an LD_{50} of 1260 mg/kg of body weight. No changes were observed in peripheral blood constituents. However, significant reductions were found in the spleen, thymus, and adrenal weights [21, 55]. Hence, *W. somnifera* can be used as safe therapeutic agent for various clinical conditions.

Pharmacokinetic studies

Numerous studies have been carried out in different biological models to elucidate the pharmacokinetics of *W. somnifera*. Two major constituents—withaferin-A and withanolide-A have been observed after oral administration of standardized *W. somnifera* aqueous extract in mice using multiple reaction monitoring. A dose of 1000 mg/kg extract (equivalent to 0.4585 mg/kg of withaferin-A and 0.4785 mg/kg of withanolide-A) demonstrated almost similar pharmacokinetic patterns for both of these withanolides with mean plasma concentrations (C_{max}) of 16.69 ± 4.02 and 26.59 ± 4.47 ng/ml for withaferin-A and withanolide-A, with T_{max} (time taken to reach C_{max}) of 10 and 20 min, respectively, indicating their rapid absorption. The area under the plasma concentration–time curve from 0 to 4 h ($AUC_{0–4h}$) was 1572.27 ± 57.80 and 2458.47 ± 212.72 min ng/ml, respectively. The $T_{1/2}$ of 59.92 ± 15.90 min and 45.22 ± 9.95 min and clearance of 274.10 ± 9.10 and 191.10 ± 16.74 ml/min/kg for withaferin-A and withanolide-A, respectively, were observed. Overall relative oral bioavailability has been found to be 1.44 times greater for withaferin-A compared to withanolide-A [56]. In addition, Thaiparambil et al. [57] have shown that withaferin-A reaches peak concentrations up to 2 μM in plasma with a half-life of 1.36 h following a single 4 mg/kg dose in 7–8-week-old female Balb/C mice, whereas the clearance from plasma is rapid (0.151 ng/ml/min). Another study has demonstrated that at a single oral dose of 500 mg/kg in six healthy buffalo calves resulted in a mean peak plasma concentration at 0.75 h and was 248.16 ± 16.12 μg/ml. Further on, the mean plasma concentration of 6.55 ± 0.12 μg/ml was detected up to 3 h. The mean therapeutic concentration (≥0.1 mg/ml) of *W. somnifera* has been maintained from 10 min to 3 h in plasma of healthy buffalo calves. The mean elimination half-life ($t_{1/2}$) of *W. somnifera* was observed to be 0.92 ± 0.032 h. The total body clearance ranges from 2.26 to 3.09 l/kg/h with a mean value of 2.78 ± 0.12 l/kg/h [58]. In a study involving Albino rabbits (1.5–1.8 kg, either sex, $n = 6$) that were fasted overnight, a single oral dose of 0.42 g/kg. *W. somnifera* (obtained from two sources) was well absorbed with a peak plasma concentration (C_{max}) of 18,317.8–21,360.7 ng/ml with a T_{max} of 1–2 h. The biological half-life ranged from 18.29 to 27.69 h [59].

Anti-microbial activity

Consistent with the folkloric use of *W. somnifera* against infections, methanolic leaf extract of *W. somnifera* has shown marked anti-bacterial activity against Gram-positive clinical isolates of methicillin-resistant *Staphylococcus aureus* and *Enterococcus* spp. [60]. Additionally, *W. somnifera* demonstrated potent anti-microbial activities against Gram-negative species such as *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* [61, 62]. The potency of *W. somnifera* has been observed to vary in different studies against different organisms. The mechanism of anti-microbial activity was ascribed to cytotoxicity, gene silencing, and immunopotentiation [63]. *W. somnifera* has strong anti-*Salmonella typhimurium* activity in vitro [62]. Additionally, increased survival rate and reduced bacterial load of various vital organs of mice with salmonellosis has been reported after administration of *W. somnifera* [64]. *W. somnifera* extracts synergized increase the anti-bacterial effect of Tibrim (rifampicin and isoniazid) against *Salmonella typhimurium* and *E. coli* [65].

W. somnifera inhibited acid production, acid tolerance, and biofilm formation of oral bacteria, *Streptococcus mutans*, and *Streptococcus sobrinus* at even sub-minimum

inhibitory concentration (MIC) levels. There was also a dose-related increase in doubling times of *Streptococcus mutans* and *Streptococcus sobrinus* up to 258 and 400 %, respectively [66]. Withanolides induces apoptosis-like death in *Leishmania donovani* in vitro by provoking DNA nicks, cell cycle arrest at the sub G_0/G_1 phase, and externalization of phosphatidylserine in a dose- as well as time-dependent manner through an increase in reactive oxygen species (ROS) and a decrease in mitochondrial potential [67] by blocking the protein kinase-C signaling pathway [68]. Importantly, anti-leishmanial activity was exhibited by *W. somnifera* against free-living promastigotes and intracellular amastigotes of Leishmania major with a maximum inhibitory effect of >50 % [69]. *W. somnifera* synergized protection in cisplatin-treated *L. donovani*-infected mice as compared to only *W. somnifera*-treated *L. donovani*-infected mice by enhancing the percentage of T cells (CD4+, CD8+) and natural killer cell-associated marker (NK1) [70]. *W. somnifera* dose-dependently reduced parasite load and protected packed cell volume drop effect in mice infected with malarial parasite. Maximum inhibition was seen at 600 mg/kg [71], while it produced a non-significant suppression (21 %) against a chloroquine-resistant *Plasmodium berghei* in mice [72].

A glycoprotein from *W. somnifera* exerts a fungistatic effect in phytopathogenic fungi by inhibiting spore germination and hyphal growth in the tested fungi *Aspergillus flavus*, *Fusarium oxysporum* and *Fusarium verticilloides* [73]. Furthermore, flavonoids extracted from *W. somnifera* have been reported to be effective against *Candida albicans* with MIC of 0.039 and minimum fungicidal concentration (MFC) of 0.039. Moreover, it was demonstrated that *A. flavus* and *Aspergillus niger* were resistant to *W. somnifera* [61].

Anti-inflammatory activity

Withania somnifera has exhibited marked anti-inflammatory effects in various disease models. Its root extract exhibited anti-inflammatory and muco-restorative activity by resolving necrosis, edema, neutrophil infiltration in trinitro-benzyl-sulfonic acid (TNBS) -induced inflammatory bowel disease [74]. Powder of its roots was found to have a potent inhibitory effect on proteinuria, nephritis, and other inflammatory markers such as cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-α, nitric oxide (NO), and ROS in a mouse model of lupus [75, 76].

In human umbilical vein endothelial cells (HUVECs), withaferin-A was shown to inhibit phorbol-12-myristate-13-acetate (PMA)-induced shedding of endothelial cell protein-C -receptor (EPCR) by inhibiting TNF-α and interleukin (IL)-1β. Moreover, in mouse withaferin-A attenuated cecal ligation and puncture (CLP)-induced EPCR shedding by reducing the expression and activity of tumor necrosis factor-α (TNF-α) converting enzyme. Additionally, withaferin-A attenuated PMA-stimulated phosphorylation of p38, extracellular regulated kinases (ERK)-1/2, and c-Jun N-terminal kinase (JNK) [77]. Withaferin-A protects vascular barrier integrity in HUVECs and in mice, induced by high mobility group box-1-protein (HMGB1) by inhibiting hyperpermeability, expression of cell adhesion molecules (CAM)s, adhesion and migration of leukocytes, production of interleukin-6, TNF-α, and activation of nuclear factor κ-β (NFκ-β) [78]. Withaferin-A prevents Iκ-β phosphorylation and degradation, which subsequently blocks NFκ-β translocation, NFκ-β/DNA binding, and gene transcription in Murine fibrosarcoma L929sA cells and human embryonic kidney 293T cells [79]. It also inhibits TNF-α-induced expression of cell adhesion molecules by inactivation of AKT and NFκ-β in human pulmonary epithelial cells [80]. Additionally, withaferin-A hampers NFκ-β activation by targeting cysteine 179 located in catalytic site of IKK-β [81]. In cellular models of cystic fibrosis, Withaferin-A leads to inhibition of NFκ-β and IL-8 [82].

Anti-arthritic activity

Ample precedent suggests a major role for *W. somnifera* in arthritis. Aqueous extracts of *W. somnifera* root powder showed a transitory chondroprotective effect on damaged human osteoarthritic cartilage by significant and reproducible inhibition of the gelatinase activity of collagenase type-2 enzyme in vitro [83] and by significantly decreased NO release [84]. Additionally, the crude ethanol extract of *W. somnifera* significantly suppressed lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-12p40 in peripheral blood mononuclear cells from normal individuals and synovial fluid mononuclear cells from rheumatoid arthritis patients possibly by inhibiting nuclear translocation of the transcription factors NFκ-β and activator protein-1 (AP-1) and phosphorylation of Iκ-β as evidenced from mouse cell line data from the same study. Additionally, it normalized LPS-induced NO production in RAW 264.7 cells [85]. In a rat model of adjuvant-induced arthritis, *W. somnifera* root powder attenuated cartilage degradation as assessed by estimation of bone collagen [86]. Aqueous extract of *W. somnifera* root prevented increased arthritic index, autoantibodies, and C-reactive-protein-P in collagen-induced arthritic rats [87]. Administration of *W. somnifera* root powder to the arthritic rats significantly decreased the severity of arthritis by effectively improving the functional recovery of motor activity and radiological score [88]. Furthermore, *W.*

somnifera as a constituent in a polyherbal formulation (BV-9238) reduced TNF-α and NO production, without any cytotoxic effects in Freund's complete adjuvant-induced arthritis in rats and a mouse macrophage cell line [89]. More importantly, *W. somnifera* helps collagen stabilization by inhibiting collagenase [90].

Some studies have reported conflictual reports regarding Withaferin-A. In rabbit articular chondrocytes, Withaferin-A-induced loss of type collagen expression and inflammatory responses mediated up-regulation of cyclooxygenase-2 (COX-2) expression through activation of microRNA-25 [91, 92]. Moreover, marked exacerbation in the production of intracellular ROS accompanied by apoptosis and increased p53 expression were observed, and these effects were dependent on PI3 K/AKT and JNK pathways [92, 93].

Anti-cancer activity

Various types of cancers or cancer-related changes in cell lines have been attenuated by *W. somnifera* or its chemical constituents. Molecular docking analysis demonstrated the use of withaferin-A and withanone for cancer drug development [94]. Leaf extract of *W. somnifera* and its components kills cancer cells by at least five different pathways—p53 signaling, granulocyte–macrophage colony-stimulating factor (GM-CFS) signaling, death receptor signaling, apoptosis signaling and by G2-M DNA damage regulation pathway [95]. Withaferin-A exhibited anti-cancer activity by inducing ROS-induced apoptosis in melanoma cells by crashing Bcl-2/Bax and Bcl-2/Bim ratios. This apoptotic cascade employed the mitochondrial pathway and was associated with Bcl-2 down-regulation, translocation of Bax to the mitochondrial membrane, release of cytochrome-c into the cytosol, abrogation of transmembrane potential, and activation of caspases-9 and 3. The withanolide-induced early ROS generation and mitochondrial membrane potential disturbances followed by the release of cytochrome c, translocation of Bax to mitochondria, and apoptosis-inducing factor to cell nuclei. These events paralleled activation of caspases-9 and 3, Poly-(ADP-Ribose) Polymerase (PARP) fragmentation of DNA [96]. Withaferin-A also led to the overexpression of tumor necrosis factor receptor (TNFR)-1 and obliterated the expression of Bid. More importantly, withaferin-A blocked binding of NFκ-β to DNA and instigated nuclear cleavage of p65/Rel by activated caspase-3. These studies suggest that withaferin-A kills cancerous cells by apoptosis that can be dependent and/or independent of mitochondrial mechanisms [97]. Enhanced production of ROS, down-regulation of Bcl-2, cleavage of PARP, stimulation of caspase-3, and mitogen-activated protein kinase (MAPK) signaling cascade are critically involved in the apoptosis induced by withaferin-A and radiation in human lymphoma U937 cells [98]. However, MAPK has a cell line-specific role in cell death by withaferin-A [99]. Similarly, Withaferin-A exacerbated radiation-induced apoptosis in human renal cancer cells by excessive generation of ROS, and by inhibition of Bcl-2 and dephosphorylation of AKT [100], and by endoplasmic reticulum (ER) stress [101]. Development of mammary cancer in a transgenic mouse model was markedly inhibited by withaferin-A by reducing the population of breast cancer stem cells and tumor size and tumor area. Similarly, mammosphere formation was dose-dependently blocked by withaferin-A treatment in human breast cancer cells which accompanied induction of apoptosis and mitigation of complex-III activity [102–104]. All these effects are independent of autophagy [105]. However, it activates Notch-2 and Notch-4, which leads to the arrest of their migration [106]. Additionally, withaferin-A causes G2 and M phase cellcycle arrest in human breast cancer cells [107]. Nagalingam et al. [108] demonstrated that Withaferin-A application inhibited breast tumor progression in xenograft and transgenic mouse models that employed up-regulation of the ERK/RSK axis, activation of Death Receptor 5 (DR5), and high levels of nuclear ETS domain-containing protein-1 (Elk-1) and CAAT/enhancer-binding protein-homologous protein (CHOP). Withaferin-A treatment inhibits experimental mammary cancer growth through the suppression of vimentin protein expression [109] by interfering with β-tubulin of cytoskeletal architecture [110].

W. somnifera killed human laryngeal carcinoma Hep2 cells and led to the arrest of the cell cycle with concomitant blockade of angiogenesis [111]. Withaferin-A inhibits cell proliferation in human umbilical vein endothelial cell inhibition of cyclin-D1 expression and by ubiquitination of proteins and defects in ubiquitin-mediated proteasome pathway [112]. Similarly, withaferin-A inhibits the growth of patient-derived mesothelioma by inhibiting proteasome and by inducing apoptosis [113].

In a kidney cancer cell line, Withaferin-A induced dose-dependent apoptotic cell death and PARP cleavage through down-regulation of the STAT-3 pathway [101, 114]. Additionally, Choi et al. [101] demonstrated that this cell death was due to ER stress. They observed that Withaferin-A led to phosphorylation of eukaryotic initiation factor-2α (eIF-2 α), ER stress-specific X-box binding protein-1 (XBP-1) splicing, and up-regulation of glucose-regulated protein (GRP)-78 and that of CHOP.

Cardio-protective activity

Withania somnifera possesses cardio-protective activity [115]. *W. somnifera* demonstrated cardiotropic and cardio-protective properties in animal models [116, 117].

Polyherbal formulations which had *W. somnifera* as a component showed cardioprotection in animal models [118, 119] by activating nuclear factor-erythroid-2-related transcription factor (Nrf)-2, stimulating phase-II detoxification enzymes, abrogating apoptosis in a Nrf-2-dependent manner [120]. Furthermore, it improved hematopoiesis [121]. Prophylactic treatment with *W. somnifera* markedly restored the myocardial oxidant/anti-oxidant balance, anti-apoptotic/pro-apoptotic effects, and reduced TUNEL positivity and lessened histopathologic deterioration of myocardium in a rat model of coronary artery occlusion [122]. These effects were in addition to restoring oxidant/anti-oxidant balance [123–125]. Similarly, standardized extract of *W. somnifera* prevented doxorubicin-induced cardiotoxicity and restoration of biochemical changes [126].

Anti-diabetic activity

Various polyherbal formulations (Dianix, Trasina) of Indian Systems of Medicine showed strong anti-diabetic activity in human subjects [127–129]. In patients, *W. somnifera* root powder stabilized blood glucose that was comparable to that of an oral hypoglycemic drug daonil, when treated orally for 30 days [130]. Additionally, *W. somnifera* treatment significantly improved insulin sensitivity index and blocked the rise in homeostasis model assessment of insulin resistance in non-insulin-dependent diabetes mellitus in rats [131]. In agreement with these studies, *W. somnifera* leaf and root extracts improved glucose uptake in skeletal myotubes and adipocytes in a dose-dependent manner, with the leaf extract demonstrating more pronounced effects than the root extract [132]. Root and leaf extracts significantly normalized the levels of urine sugar, blood glucose, glucose-6-phosphatase, and tissue glycogen levels in alloxan-induced diabetes mellitus in rats. Additionally, attenuation of improving the non-enzymatic and enzymatic anti-oxidant defenses was also observed [133, 134]. Withaferin-A blocks inflammatory response in cytokine-induced damage to islets in culture and following transplantation [135] and exhibits potent anti-glycating activity [136].

Anti-stress activity

Withania somnifera resulted in better stress tolerance in animals [137–139]. The aqueous fraction of *W. somnifera* roots alleviated chronic stress-induced reduction of T cell population and up-regulated Th1 cytokines in mice [140]. In a clinical study for the safety and efficacy of a high-concentration full-spectrum extract of *W. somnifera* roots in human subjects, serum cortisol levels were reduced, without causing any major side effects [141]. Furthermore, EuMil, a poly herbal formulation markedly ameliorated cerebral monoamine (nor-adrenaline, dopamine, and 5-hydroxytryptamine) levels induced by chronic electroshock stress [142]. In another study, EuMil restored chronic stress-induced glucose intolerance and normalized male sexual behavior and behavioral despair. Additionally, it attenuated cognitive dysfunction, immunosuppression, gastric ulceration, and plasma corticosterone levels [143]. Another poly-herbal formulation (Perment®) exhibited anti-depressant and anxiolytic activity in rats, which was partly due to activation of adrenergic and serotonergic systems [144]. Glycowithanolides from *W. somnifera* produced an anxiolytic effect against pentylenetetrazole-induced anxiety in rats, which was comparable to that exhibited by well-known anti-depressants. In addition, it reduced rat brain levels of tribulin, an endocoid marker of clinical anxiety [145]. Further on, it normalized oxidative free radical scavenging enzymes and lipid peroxidation (LPO) in rat frontal cortex and striatum of chronically footshock stressed rats [146].

Neuroprotective activities

Many studies have documented the neuroprotective effects of *W. somnifera* [22, 147–150]. The leaf extract and its component withanone protect scopolamine-induced toxic changes in both neuronal and glial cells. Scopolamine-induced inactivation of neuronal cell markers such as NF-H, MAP-2, PSD-95, GAP-43, and glial cell marker glial fibrillary acidic protein (GFAP) and with DNA damage and oxidative stress markers was markedly attenuated by *W. somnifera* [151]. *W. somnifera* extract attenuated lead-induced toxicity in glial cells by balancing the expression of GFAP and heat shock protein (HSP70), mortalin, and neural cell adhesion molecule (NCAM) [152]. Glycowithanolides from *W. somnifera* exhibited significant anti-oxidant activity in cortex and striatum of rat brain by inducing a dose-related increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity [153]. Additionally, extract of *W. somnifera* prevented streptozotocin-induced oxidative damage in treated mice by mitigating oxidative stress [154]. *W. somnifera* root powder extract markedly rescued the number of degenerating cells in CA2 and CA3 sub-areas of rats hippocampus subjected to immobilization stress [155]. *W. somnifera* root extract or its derivatives promoted neurite outgrowth extensions in human neuroblastoma cell lines [156]. The axons are mainly extended by withanolide-A, and dendrites by withanolides-IV and VI while withanoside-IV induced both axonal and dendritic rejuvenation and synaptic restoration in rat cortical neurons damaged by

amyloid-β (Aβ) [157, 158]. Kataria et al. [159] demonstrated that *W. somnifera* leaf extract rescued retinoic acid-differentiated C6 and IMR-32 cells from glutamate toxicity. *W. somnifera* leaf extract pre-treatment inhibited glutamate-induced cell death and reversed glutamate-evoked stress response by up-regulation of HSP70 and additionally it restored neuronal plasticity by neuronal plasticity markers, neural cell adhesion molecules, and its polysialylated form. *W. somnifera* extract also reduced kainic acid-induced excitotoxic damage by mitigating oxidative stress [160].

Anti-Parkinson activity

Precedent exists in literature for a major role for *W. somnifera* in Parkinson's disease. *W. somnifera* have been shown to attenuate Parkinson symptoms and pathology in a 6-hydroxydopamine (6-OHDA) rat model for the disease. The study demonstrated the restoration of the content of striatal dopamine and its metabolites most likely via its pronounced anti-oxidant action as evidenced by the attenuation of LPO, reduced glutathione (GSH) content, and activities of glutathione-*S*-transferase (GST), glutathione reductase (GR), GPX, SOD, and CAT. Improvement of striatal catecholamine content due to *W. somnifera* might have reversed the functional impairments like locomotor activity and muscular coordination and drug-induced rotational behavior. This study also demonstrated up-regulation of dopaminergic D2 receptor populations in striatum, which acts as a compensatory mechanism after induction of Parkinsonism to grab every available dopamine molecule. Additionally, *W. somnifera* has led to an increase in the number of surviving dopaminergic neurons as estimated by tyrosine hydroxylase labeling [161]. *W. somnifera* root extract restored anti-oxidant status, reduced oxidant stress, and thus normalized catecholamine content in mid brain of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated parkinsonian mice. These biochemical changes accompanied the betterment in functional activity of the model [162–164]. Standardized extract of *W. somnifera* significantly reduced rotenone-induced oxidative impairment and mitochondrial respiratory chain enzymes that in turn have attenuated disturbances in cholinergic function and repleted dopamine content. These changes were responsible for reduced locomotor deficits and lethality in a *Drosophila melanogaster* model of Parkinson induced by rotenone [165]. Additionally, rotenone toxicity in cerebellum and striatum of mouse brain was greatly decreased by *W. somnifera* root powder through its anti-oxidant and anti-inflammatory actions and by correcting mitochondrial dysfunctions. These changes brought about restoration of neurotransmitter functions and dopamine levels in striatum [166]. Maneb-paraquat-induced mouse model of Parkinson and ethanolic root extract of *W. somnifera* rescued dopaminergic neurons as measured by expression of tyrosine hydroxylase, replenished dopamine levels in the substantia nigra, and attenuated locomotor activity by reducing inflammation and apoptosis and various aspects of oxidative damage. In particular, *W. somnifera* reduced the expression of inducible NO synthase (iNOS), a measure of oxidative stress. *W. somnifera* deactivated pro-apoptotic Bax and activated anti-apoptotic Bcl-2 protein expression and down-regulated the activation of astrocytes and expression of GFAP [167, 168].

Anti-Alzheimer activity

Literature suggests a prominent role of *W. somnifera* in drug development against Alzheimer's disease. Standardized aqueous extract of *W. somnifera* improved cognitive and psychomotor performance in healthy human participants [169]. *W. somnifera* root extract reversed the behavioral deficits and pathological clues as well as Aβ clearance in Alzheimer's disease models by up-regulating lipoprotein receptor-related protein in liver [170]. Simulation studies have shown that withanamides-A and -C uniquely bind to the active motif of $(A\beta)_{(25–35)}$ and suggest that withanamides have the ability to prevent the fibril formation and thus protect cells from Aβ toxicity [171]. Furthermore, docking simulation studies have predicted inhibition of human acetyl cholinesterase by withanolide-A for Alzheimer's treatment [172]. Withanoside-IV and its active metabolite, sominone, attenuated $A\beta_{(25–35)}$-induced neurodegeneration by improving memory deficits in mice and preventing a loss of axons, dendrites, and synapses [158]. *W. somnifera* elicits a protective response and abolishes acetylcholine esterase (AChE) activity inhibition and cognitive impairment caused by subchronic exposure to propoxur to rats [173]. *W. somnifera* affords a beneficial effect on cognitive deficit by ameliorating oxidative damage induced by streptozotocin in a model of cognitive impairment [174]. *W.* somnifera restored cellular morphology in Aβ-treated SK-N-MC cell line by enhancing cell viability and the peroxisome proliferator-activated receptor-γ (PPAR-γ) levels [175]. Further on, it led to inhibition of acetylcholinesterase activity [176]. *W. somnifera* root extract concentration dependently exhibited protective effects against hydrogen peroxide and $A\beta_{(1–42)}$-induced cytotoxicity in differentiated PC12 cells [177].

Anti-ischemic and anti-hypoxic activity

Withania somnifera attenuated middle cerebral artery occlusion-induced enhancement of the oxidative stress marker malondialdehyde, reduction in lesion area, and

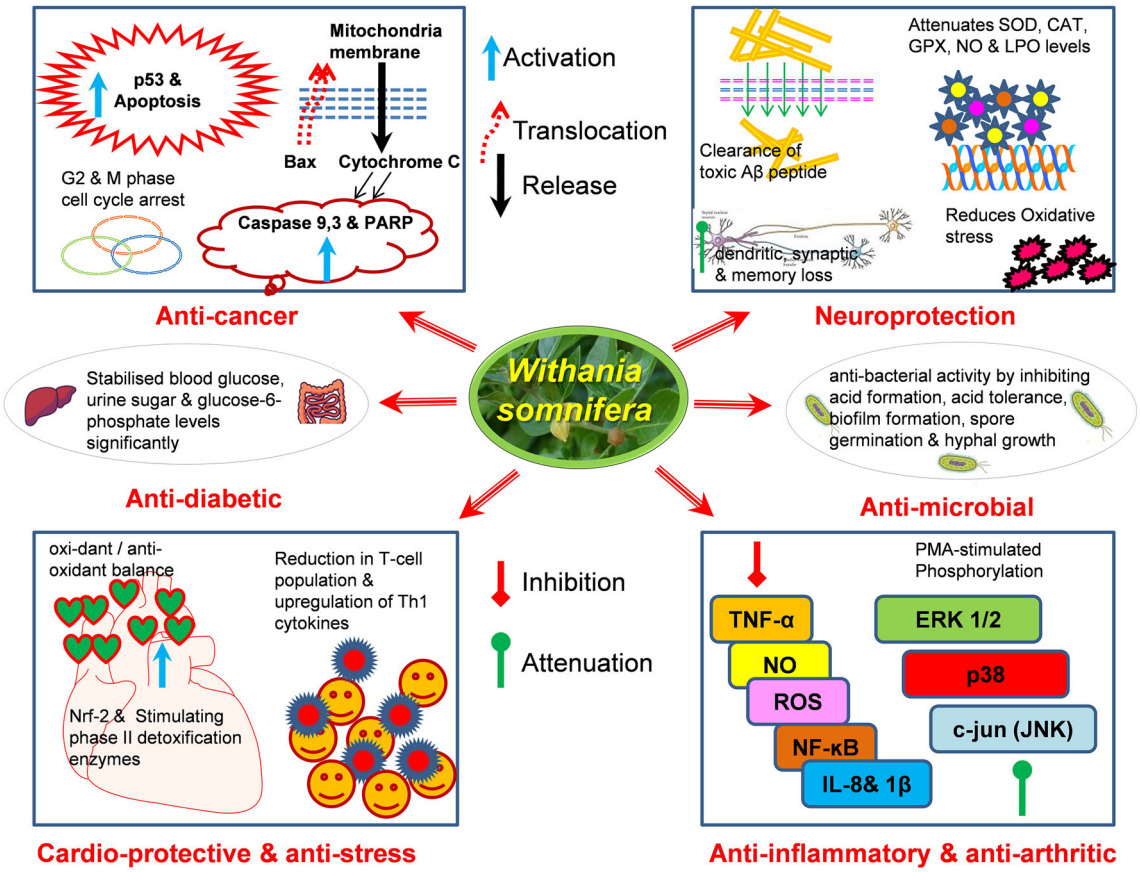

Fig. 1 *Withania somnifera* exerts multiple pharmacologic actions such as neuroprotection (reducing oxidative stress by restoring antioxidant levels, clearance of Aβ levels, attenuating synaptic and dendritic loss and reversing SOD, CAT, GPx, NO and LPO levels), cardio-protection (anti-oxidant balance and activating Nrf-2 and stimulating phase-II detoxification enzymes) anti-inflammatory, anti-oxidant and anti-stress (inhibiting NFκ-β transcription, MAPK signaling pathways, TNF-α, NO, ROS and IL-8, reducing T-cell population and up-regulating Th1 cytokines), anti-diabetic (stabilizing blood glucose, urine sugar, and glucose-6-phosphate levels significantly), anti-bacterial (inhibiting acid formation, acid tolerance, biofilm formation, spore germination, and hyphal growth) and anti-cancer (cell cycle arrest and activation of p53, loss of mitochondrial membrane potential, activation of caspase cascade and PARP-1)

restoration of neurological deficits [178]. *W. somnifera* imparted functional restoration and attenuation of infarct volume in mice subjected to permanent distal middle cerebral artery occlusion (pMCAO). It led to recovery of hemeoxygenase-1 (HO-) expression and abated the up-regulation of the proapoptotic protein PARP-1 via the PARP-1 apoptosis-inducing factor (AIF) pathway that was altered by pMCAO in mouse cortex. This phenomenon led to a blockade of the apoptotic cascade by preventing nuclear translocation of AIF. Additionally, semaphorin-3A expression was increased by pMCAO and it initiates inhibitory signals that thwart repair. *W. somnifera* significantly reduced the expression of Semaphorin-3A and thus initiated repair mechanisms [179, 180]. *W. somnifera* root extract and withanolide-A attenuated hypobaric hypoxia-induced memory and hippocampal neurodegeneration by repleting reduced glutathione (GSH) levels through activation of the glutathione biosynthesis pathway in hippocampal cells. These effects were mediated by the Nrf-2 pathway and NO in a corticosterone-dependent manner [181, 182].

Conclusions

Withania somnifera is a natural product with promising pharmacological and pharmaceutical properties; it has extensive clinical applications in Indian Systems of Medicine (Fig. 1). In animal studies, it or its constituents exert multiple protective properties such as anti-inflammatory, anti-oxidant, inhibiting NFκ-β transcription, MAPK signaling pathways, anti-apoptotic, angiogenic, and ER stress reducing effects (Table 2). The claims for use of *W. somnifera* to improve a myriad of clinical conditions are

Table 2 Various pharmacological action of *W. somnifera* or its chemical constituents

W. somnifera	Dosage and mode of administration	Diseased condition/model	Mechanism of action	References
Methanolic leaf extract	2 mg/ml in vitro	Methicillin resistant *Staphylococcus aureus* and *Enterococcus* spp.	Anti-bacterial activity	[60]
Methanolic extract	0.125–2 mg/ml in vitro	Oral infections by *Streptococcus mutans* and *Streptococcus sobrinus*	Inhibited acid production, acid tolerance, and biofilm formation of oral bacteria	[66]
Withanolides (F5 and F6 fractions)	60 and 15 μg/ml in vitro	*Leishmania donovani*	Apoptosis, DNA nicks, cell cycle arrest and externalization of phosphatidylserine, increased ROS, and decreased mitochondrial potential	[67]
Glycoproteins	20 μg/ml in vitro	*Aspergillus flavus*, *Fusarium oxysporum, F. verticilloides*	Inhibiting spore germination and hyphal growth	[73]
Root extract	500 μg/ml and 1000 mg/kg b.wt. (rectal route)	TNBS-induced inflammatory bowel disease in rats	Mucorestorative and anti-inflammatory, resolved edema, neutrophil infiltration and necrosis	[74–76]
	500 and 1000 mg/kg b.wt. (orally)	Mouse model of lupus	Inhibited proteinuria, nephritis, TNF-α, NO and ROS	
Withaferin-A	1.882 μg per mouse (I.V.)	Human umbilical vein endothelial cells	Inhibited TNF-α and IL-1β	[77, 79, 82]
	2 μM in vitro	Murine fibrosarcoma	Attenuated p38, ERK-1/2, C jun JNK	
	3 μM/ml in vitro	Cellular models of Cystic Fibrosis inflammation (KKLEB cells)	Inhibited Iκβ phosphorylation and degradation by blocking NFκ-β translocation, inhibited IL-8	
Aqueous extract of root powder	10 mg/ml in vitro	Human osteoarthritis (cartilage damage explant models)	Chondroprotective actions by inhibiting gelatinase activity of collagenase type-2 enzyme and decreased NO	[83, 88]
	600 and 800 mg/kg (orally)	Collagen-induced arthritis in rats	Attenuated cartilage degradation, improved the functional recovery of motor activity and radiological score	
Crude ethanolic extract	1 mg/ml in vitro	Rheumatoid arthritis (PBM cells)	Suppression of LPS-induced production of cytokines, interleukins, and TNF-α	[85]
Leaf extract	6, 15, 21, 25, and 32 μg/ml in vitro	Cancer cells (TIG1, U2OS, and HT1080)	Activated p53, apoptosis pathway, and arresting cell cycle	[95]
Withaferin-A	3 μM/ml in vitro	Human melanoma cells (M14, Lu1205, and Sk28)	Promoted ROS-induced apoptosis by lowering Bax/Bcl2 and Bcl2/Bim ratio	[96, 107, 108, 111]
	2 and 3 μM/ml in vitro	Breast cancer cells (MDA-MB-231 and MCF-7)	Translocation of Bax to mitochondrial membrane resulting in cytochrome c release and activation of caspase-9 and 3 and PARP	
	5 and 10 μM/L in vitro and injections of 4 mg/kg b.wt. 5 days/week for 5 weeks. (i.p.)	Breast tumor progression in xenograft and transgenic mouse models	G2 and M-phase cell cycle arrest, up-regulated ERK/RSK axis, activation of DR-5, Elk1 and CHOP	
	25 μg/ml in vitro	Human laryngeal carcinoma Hep2 cells	Cell cycle arrest with concomitant blockade of angiogenesis	
	4 μM/ml in vitro	Renal cancers (Caki cells)	PARP cleavage through down-regulation of STAT-3 pathway	[101, 114]
	26 μM/ml in vitro		ER stress-specific XBP1 splicing, and up-regulation of GRP-78 and CHOP	

Table 2 continued

W. somnifera	Dosage and mode of administration	Diseased condition/model	Mechanism of action	References
Whole extract	30, 60 and 90 mg/kg/day for 60 days (orally)	Myocardial infarction in rats	Cardiotropic and cardioprotective	[116, 117, 120, 122, 125]
	50, 75 and 100 mg/ml in vitro	Coronary artery occlusion in rats	Activated Nrf2, stimulated phase II detoxification enzymes, abrogated apoptosis in a Nrf2-dependent manner	
	50 mg/kg b.wt. for 30 days (orally)		Anti-apoptotic/pro-apoptotic effects, and reduced TUNEL positivity and lessened histopathologic deterioration of myocardium	
Root extract	3 g/day human subjects (orally)	Diabetes	Stabilized blood glucose levels	[130, 131, 133, 134]
Aqueous extract	200 and 400 mg/kg b.wt./day for 5 weeks (orally)	Non-insulin-dependent diabetes mellitus in rats	Improved insulin sensitivity index and blocked the rise in homeostasis model assessment of insulin resistance	
Root and leaf extract	200 mg/kg b.wt. for 8 weeks(orally)	Alloxan-induced diabetes mellitus in rats	Normalized the urine sugar, blood glucose, glucose-6-phosphatase and tissue glycogen levels	
Aqueous fraction of roots	25, 50, 100 and 200 mg/kg for 14 days (orally)	Mouse model of chronic stress	Reduced in T-cell population and up-regulated Th1 cytokines	[140]
EuMil, poly herbal formulation	100 mg/kg for 14 days(orally)	Chronic electroshock stress in rats	Ameliorated cerebral monoamine levels	[142, 143]
	100 mg/kg for 14 days (orally)		Attenuated cognitive dysfunction, immunosuppression, gastric ulceration, and plasma corticosterone levels	
Glycowithanolides	20 and 50 mg/kg for 5 days (orally)	Pentylenetetrazole induced anxiety in rats	Anxiolytic effects and reduced rat brain levels of tribulin	[145]
Leaf extract and Withanone	100, 200 and 300 mg/kg b.wt. for 7 days (orally)	Scopolamine induced toxicity in mice	Produced neuronal and glial protection cells by activating neuronal proteins, oxidative stress and DNA damage	[151]
Root extract	20 mg/kg b.wt. for 30 days (orally)	Immobilization stress in albino rats	Markedly rescued the number of degenerating cells in CA2 and CA3 subareas of rat hippocampus	[155]
Withanolide-A, withanolides-IV, Withanoside-VI	10 μM/kg/day (orally)	Amyloid- β toxicity (rat cortical neurons)	Promoted neurite outgrowth, axonal and dendritic and synaptic rejuvenation	[157, 158]
Water extract	0.05 and 0.1 % in vitro	Glutamate induced excitotoxicity in IMR-32 and C6 cells	Reversed glutamate-evoked stress response by up-regulation of HSP70, restored neuronal plasticity, reduced kainic acid-induced excitotoxic damage by mitigating oxidative stress	[159, 160]
Whole extract	100, 200 and 300 mg/kg b.wt. for 3 weeks (orally)	6-OHDA induced toxicity in rats	Attenuated lipid peroxidation, reduced glutathione content, and activities of glutathione-*S*-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase, increased number of dopaminergic neurons	[161]
Root extract	100 mg/kg b.wt. (orally)	MPTP induced toxicity in mice	Normalized catecholamine content, reduced oxidant stress, and functional activity	[162, 164]
Root powder	100 and 400 mg/kg b.wt./day for 4 weeks (orally)	Rotenone-induced impairment in mice	Antioxidant and anti-inflammatory actions and corrected mitochondrial dysfunctions, normalized neurotransmitter function, and dopamine levels in striatum	[166]
Ethanolic extract	100 mg/kg b.wt. for 3, 6, and 9 weeks (orally)	MBPQ-induced toxicity in mice	Rescued dopaminergic neurons, replenished dopamine levels in substantia nigra and attenuated locomotor activity and reduced oxidative stress and inflammation	[167, 168]
	100 mg/kg b.wt. for 9 weeks(i.p)		Activated anti-apoptotic Bcl-2 protein expression and down-regulated pro-apoptotic Bax and astrocytes and expression of GFAP	

Table 2 continued

W. somnifera	Dosage and mode of administration	Diseased condition/model	Mechanism of action	References
Standardized aqueous extract	250 mg twice daily for 14 days to human subjects (orally)	Psychomotor functional disorders in healthy humans	Improved cognitive and psychomotor performance	[169]
Root extract	1 g/kg b.wt. for 7–30 days (orally)	Alzheimer's disease models	Reversed the behavioral deficits and pathological clues as well as Aβ clearance by up-regulating lipoprotein receptor-related protein in liver	[170]
Withanolides	6.25, 12.5, 25, 50, 100 μg/ml in vitro	Alzheimer's disease transgenic mice	Prevented the fibril formation and thus protect cells from amyloid- β toxicity	[171]
Withanoside-IV and Sominone	10 μM/kg/day (orally)	Alzheimer's disease mice	Attenuated $A\beta_{(25,35)}$ induced neurodegeneration and improved memory deficits in mice and prevented loss of axons, dendrites, and synapses	[158]
Whole extract	0.15 and 0.3 μg/ml in vitro	Aβ toxicity in SK-N-MC cells	Enhanced cell viability and PPARγ levels, inhibited of acetyl- cholinesterase activity	[175, 176]
Whole extract	1 g/kg for 15 and 30 days (orally)	Middle cerebral artery occlusion in rats	Attenuated oxidative stress marker malondialdehyde, reduced lesion area, and restoration of neurological deficits	[178]
Root extract	50, 100, 150, 200 and 250 mg/kg b.wt. for 21 days (orally)	Hypoxia pathway in hippocampal cells	Enhanced memory and attenuated hippocampal neurodegeneration by repleting glutathione levels through activation of glutathione biosynthesis	[181, 182]

overwhelmingly encouraging as a multi-purpose medicinal agent. More clinical validation needs to be performed for its general medical use.

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Compliance with ethical standards

Conflict of interest Authors do not have any conflict of interest.

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