RESEARCH PAPER

A CRITICAL REVIEW OF WITHANIA SOMNIFERA L. WITH SPECIAL REFERENCE TO ITS PHARMACOLOGICAL ACTIONS

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ABSTRACT

Withania somnifera L. Dunal, commonly known as Ashwagandha, is a member of the nightshade family Solanaceae. This plant holds significant pharmacological importance in the Indian subcontinent and has been a cornerstone of Ayurvedic and indigenous medicine for over 3,000 years. This review compiles the latest information on its phytochemical and pharmacological activities, derived from diverse methodologies and sources, including Ayurvedic texts and various literature. Studies have shown that Ashwagandha possesses a range of beneficial properties, such as antioxidant, anxiolytic, adaptogenic, memory-enhancing, antiparkinsonian, antivenom, anti-inflammatory and antitumor effects. Additionally, it has been studied for its immunomodulatory, hypolipidemic, antibacterial, cardiovascular protective, and sexual behavior enhancing properties, as well as its impact on tolerance and dependence. The biological activities of *Withania somnifera* L. are primarily linked to two key withanolides: Withaferin-A and Withanolide-D. Withaferin-A, in particular, has demonstrated the ability to induce apoptosis (programmed cell death) in cancer cells by disrupting the cellular mechanisms that support tumor growth and metastasis. Besides withanolides, Ashwagandha contains other bioactive constituents, including Withanosides, sitoindosides, steroidal lactones and alkaloids, all of which offer a broad spectrum of therapeutic potential. Various research groups globally have identified several molecular targets of *Withania somnifera* L., such as inhibiting the activation of nuclear factor kappa-B and promoting cancer cell apoptosis. This review underscores that *Withania somnifera* L. is a rich source of withanolides and other bioactive compounds, making it a promising candidate for treating various chronic diseases, given its minimal side effects observed in pre-clinical studies. The encouraging results suggest that Ashwagandha should be further investigated to confirm these findings and uncover additional therapeutic benefits.

Keywords: Ashwagandha; Anti-inflammatory; Antioxidant; Withaferin-A; Withanolides.

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INTRODUCTION

Withania somnifera L., also known as *Ashwagandha,* Indian ginseng and winter cherry, has been an important herb in the Ayurvedic and indigenous systems of medicine for over 3000 years. The roots of the plant are categorised as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing.

It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent, Anti Cancer and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of *Ashwagandha,* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease. A*shwagandha,* is chemo preventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. Recently *Ashwagandha,* is also used to inhibit the development of tolerance and dependence on chronic use of various psychotropic drugs.[1,2]

Withania somnifera L.*,* commonly known as Ashwagandha or Winter Cherry, is a medicinal plant classified under Kingdom *Plantae*, Division *Angiosperma*, and Family *Solanaceae*. It is a small, evergreen, woody shrub growing 30–150 cm tall, native to dry regions of India, Africa and the Mediterranean. The plant features fleshy, whitish-brown roots, ovate glabrous leaves and greenish-yellow flowers with orange-red berries enclosed in a persistent calyx. The roots, along with other plant parts like leaves, fruits, and seeds, are used in traditional

medicine. Ashwagandha is widely recognized across India with various regional names.[3]

Phytochemistry

The isolation and characterization of secondary plant metabolites are important for the development of new therapeutics to address various health conditions.[4,5] A large number of phytochemicals have been isolated and identified from *Withania somnifera L.* using various chromatographic and spectroscopic analytical techniques such as column chromatography, gas chromatography-mass spectroscopy (GC- MS), liquid chromatography-mass spectroscopy (LC-MS), nuclear magnetic resonance (NMR) and X-Ray diffraction studies.[6]

Various phytochemical studies have revealed the presence of different bioactive constituents from various parts of *Withania somnifera* L.. Several preliminary phytochemical screenings indicated the presence of steroidal lactones, alkaloids, saponin, flavonoids, tannin, starch, phenolic content, carbohydrate, withanolides, sitoindosides, anaferine, anahygrine, *ß*-sitosterol, chlorogenic acid, cysteine, cuscohygrine, pseudotropine, withanine, scopoletin, withananine, somniferinine, somniferiene, tropanol, 14-α-hydroxywithanone and [6,7] β-Epoxywithanon isolated phytocompompouds *viz.*, withaniol, somnirol, somnitol, withanic acid, phytosterol, ipuranol and alkaloids (such as somniferine, somniferinine, withamine, withanmine, pseudowithamine, and withanaminine, etc.) from the alcoholic leaf and root extracts of *Withania somnifera* L..[7,8] The first withanolide isolated from *Withania somnifera* L. was Withaferin-A by Lavie et al.(1965).[9] Other withanolides present are Withanolide-A, Withanolide-E, Withanone, etc.[10,7] The methanolic leaf extract showed the existence of tisopelletierine,3α tigloyloxtropine, cuscohygrine, hentriacontane, visamine, etc.,[11] reducing sugars, ducitol, starch, iron and some amino acids such as glutamic acid, cysteine, tryptophan, etc.[12] In addition, steroids like cholesterol, diosgenin, stigmastadien, sitoinosides VII-X have been reported in the plant.[13] Isolated seven new withanosides glycosides *viz.*, withanosides I-VII, and four known compounds such as withaferin A, 5α,20αF (R)-dihydroxy-6α,7α-epoxy-1-oxowitha-2,24-dienolide, physagulin D and coagulin Q from the methanol root extract of *Withania somnifera* L..[14]

Molecular Targets of *Withania somnifera* L.

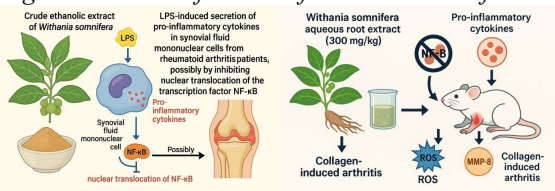
The primary bioactive constituents present in *Withania somnifera* L. are the steroidal lactones known as withanolides (withaferin A and withanolide D), which target different biomolecules in the living systems and are responsible for their diverse pharmacological activities.[15] Various *in-vivo* and *in-vitro* studies have shown that these two compounds target enzymes like kinases, growth factors, transcription factors, receptors, and structural proteins.[16,17] These have proven therapeutic potential related to the central nervous system (CNS), cardiovascular system, cancer and inflammatory and metabolic disorders.[18] The root extract of *Withania somnifera* L. has been reported to possess a potent inhibitory effect on inflammatory markers such as cytokines (IL-2 and IL-8) in mouse models.[19] Withaferin A and Withanolide D from root extract inhibited the growth of cancer cells and increased their apoptosis by causing up and downregulation of many biomolecules, such as upregulation of caspases-3, etc., which increases apoptosis in cancer cells.[20] Withaferin A suppresses cancer *via* activating tumor suppressor proteins.16 They also can act by reducing the expression of estrogen receptors that bind to endogenous estrogen to inhibit the growth of cancer cells.[21] The root extract of *Withania somnifera* L. has also been shown to mitigate memory loss in male rats by increasing reduced glutathione (GSH) levels through activation of glutathione biosynthesis in hippocampal cells showing some benefit in Alzheimer's disease.[22] Different extracts and bioactive compounds of *Withania somnifera* L. have enormous potential to target multiple biomolecules involved in the pathogenesis of various diseases, which are discussed in the following sections.

Anti-inflammatory/antiarthritic/analgesic activity

Withania somnifera L. has been found to exhibit excellent anti- inflammatory activities in several *in-vitro* and *in-vivo* models (as presented in Figure 1).[15] *Withania somnifera L.* root extract has shown decreased mRNA expression of inflammatory cytokines such as interleukin 2 (IL-2), interleukin 8 (IL-8) and tumor necrosis factor (TNF), whereas increased the mRNA expression of anti-inflammatory cytokine transforming growth factor (TGF) in a human keratinocyte cell line (HaCaT).[23,24] Furthermore, it was evaluated to have anti- inflammatory activity of the aqueous root extract of *Withania somnifera* L. topically applied to the wounded skin of 7-week-old male C57BL/6J mice for 5 days at a concentration of 10 mg/mL.[25] The results demonstrated inhibition of the pro-inflammatory cytokine TNF-α and increased anti-inflammatory cytokine TGF-β1 mRNA expression. In the wound-healing assay, the extract-treated skin showed a considerable decrease in the wound area and less immune cell aggregation than the control-treated skin. Withaferin-A is effective in treating various inflammatory conditions in diseases such as inflammation associated with arthritis, cystic fibrosis and inflammatory bowel disease by different mechanisms like inhibiting nuclear factor kappa B

(NF-κB) activation and inhibition of cyclooxygenase-2 (COX-2) generation. Withaferin-A has been shown to increase the expression of an osteoblast-specific transcription factor, which improves osteoblast differentiation and growth in menopausal osteoporosis and bone damage. [26 , 27] Withaferin-A has been shown to reduce the cecal ligation and puncture (CLP)- induced endothelial protein C receptor (EPCR) shedding by reducing the expression and activity of tumor necrosis factor-α converting enzyme in mice. Both *Withania somnifera* L. root extract and its bioactive compound (withaferin-A), downregulate the production of inflammatory mediators such as histamines, prostaglandins, and interlukins.[28,29] The aqueous root extract of *Withania somnifera* L. showed a transient chondroprotective effect on damaged human osteoarthritic cartilage by significant and reproducible inhibition of the gelatinase activity of collagenase type-2 enzyme *in-vitro* models in which explants from osteoarthritis patients were used.[29]

Fig No.1 : Mode of Action of Withania somnifera L.



The crude ethanolic extract of *Withania somnifera* L. suppressed lipopolysaccharide (LPS) induced secretion of pro-inflammatory cytokines in synovial fluid mononuclear cells from rheumatoid arthritis patients, possibly by inhibiting nuclear translocation of the transcription factor NF-κB. *Withania somnifera* L. aqueous root extract decreased the production of pro-inflammatory cytokines mediated *via* inhibition of NF-κB activity in arthritic rats. By bringing the levels of ROS and metaloproteinase-8 in rats with collagen-induced arthritis back to normal, oral administration of *Withania somnifera* L. aqueous root extract(300 mg/kg) reduced the transcription factors of arthritis in those animals.[7, 19, 29] The methanolic root extract of *Withania somnifera* L. prolonged the morphine-induced analgesia by possibly involving activation of peroxisome proliferator-activated receptor γ in male Sprague rats because the antagonist of this nuclear receptor GW-9662 attenuates the morphine-induced analgesia.[30,31]

Antiparkinson activity

Parkinson's disease (PD) has been associated with both mitochondrial dysfunction and oxidative stress. Oxidative stress affects dopaminergic neurons, cholinergic receptors, and several other structures implicated in neurodegeneration, triggering a chain of events that includes mitochondrial malfunction and neuroinflammation.4 Leucine-Rich Repeat Kinase 2 (LRRK2), also known as dardarin, is a large protein mutated in patients with familial PD, increased levels of this protein are associated with neuronal toxicity.[32,33] It is stabilized by the chaperone heat shock protein 90 (Hsp90) and its co-chaperone Cdc37. The microglial cell line N9 treated with withaferin-A reduced cellular levels of LRRK2 in a concentration and time-dependent manner, disrupting the interaction between Hsp90, Cdc37 and LRRK2, which resulted in LRRK2 instability and downregulation. Furthermore, celastrol (an inhibitor of the Hsp90-Cdc37 complex) reduced LRRK2 levels, while Withaferin-A increased LRRK2 clearance in the presence of celastrol.[34] 6-Hydroxy dopamine and 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) are used to evoke PD symptoms in animal models.[35] Oral administration of ethanolic root extract of *Withania somnifera* L. at a dose of 300 mg/kg/day improved gripping ability, motor movements and increased dopamine levels in the striatum of male Wistar rats in the 6-hydroxy dopamine-induced model, as well as maneb and paraquat models, by quenching free radicals and thereby protecting the dopaminergic neurons. Increased levels of antioxidant enzymes such as glutathione peroxidase, catalase, and glutathione reductase were observed, whereas indications of oxidative stress such as lipid peroxidation and nitrite levels were decreased.[36]

The ethanolic root extract of *Withania somnifera* L., administered at a dose of 100 mg/kg for nine weeks, was shown to provide nigrostriatal dopaminergic neuroprotection against Parkinsonism caused by maneb and paraquat through the modulation of oxidative stress. This was demonstrated to result in notable improvements in canonical PD indicators, such as impaired locomotor activity, improved pro-apoptotic state by reducing Bax and inducing Bcl-2 protein expression, decreased dopamine in the substantia nigra, decreased iNOS expression and GFAP (a pro-inflammatory marker of astrocyte activation).[37]

Anti-cancer activity

Cancer is characterized by uncontrolled cell proliferation and existing chemotherapeutic therapies target various cell signalling pathways and have a direct cytotoxic effect on cells to suppress cancer growth, but withdrawal symptoms limit their use. [38] *Withania somnifera* L. has shown a positive safety profile and remarkable anticancer potency in animal models.[39] Withaferin-A induces apoptosis in cancer cells *via* mechanisms such as inhibiting the activation of NF-κB by preventing the TNF-induced activation of IκB kinase *ß via* a thioalkylation sensitive redox mechanism, activation of tumor suppresser proteins such as p53 and pRB.[16,27] Withaferin-A has also been

shown to upregulate death receptor-5 and transduces apoptosis signal, which results in programmed cell death of cancer cells. Withaferin-A increases Par-4 induction and p38 MAP kinase activation to induce programmed cell death in cancer cells.[40] On prostate cancer cells, withaferin-A arrests the G2/M phase cell cycle and prevents mitosis by upregulation of phosphorylated Wee-1, phosphorylated histone H3, p21 and aurora B targets.[41] Withaferin-A also possesses its apoptotic action on human colon cancer cells through inhibition of the Notch-1 signalling pathway and down-regulating pro-survival pathways, such as Akt/NF-κB/Bcl-2 in three colon cancer cell lines (HCT-116, SW-480, and SW-620).[42] It also induces apoptosis in human breast cancer cells through mechanisms such as inhibition of cell migration/invasion through downregulation of signal transducer and activator of transcription (STAT3) activity.[17,27,40] Withaferin-A increases cytotoxicity in HepG2 (hepatocellular carcinoma) through upregulation of caspase-3, caspase-8 and caspase-9, which resulted in excessive programmed cell death.[17,20] Withaferin-A decreases cell survival in B cell lymphoma cell line by downregulation of heat shock protein 90 (HSP90), as this protein stabilizes several proteins required for tumor growth.[43] *Withania somnifera* L. root extract reduced cell viability and G2/M phase cell cycle arrest in a prostate cell line (PC3) by downregulation of transcription factors, which resulted in decreased biosynthesis of IL- 8 and COX-2 enzymes used 2.5 uM and 5 uM dosages of Withaferin-A on MDA-MB-231 and MCF-7 human breast cancer cells.[44] The study revealed the molecular intricacies governing Withaferin-A- mediated IAP dysregulation.[45] Survivin and cIAP-2 mRNA levels were reduced by Withaferin-A treatment, although XIAP mRNA levels were only minimally affected. The stability of cIAP-2, XIAP or Survivin mRNA was not altered, following treatment with withaferin-A, at least in the MDA-MB231 cell line. Mammary carcinogen methylnitrosourea (MNU) was used to induce breast cancer in female Sprague-Dawley rats and reported that treatment with *Withania somnifera* L. root extract at a dose of 150 mg/kg bw for 155 days decreased the number of tumours by 23% when compared to the control group.[21] Withaferin-A *via* blocking IL-6-induced STAT3 phosphorylation caused concentration-dependent apoptotic cell death in Caki human renal cells).[46] In another study, withaferin-A induced apoptosis in renal carcinoma cells mediated by endoplasmic reticulum stress, which upregulated the transcription factor CCAAT/enhancer-binding protein homologous protein (CHOP).[47]

Neuroprotective activity

Withania somnifera L. has proven to perform a neuroprotective role in various preclinical and clinical studies.[48] *Withania somnifera* L. root extract downregulated NO production by interacting with nNOS, which inhibited the stress- induced NADPH-diaphorase activation by suppressing corticosterone release and activating choline acetyltransferase which increased the serotonin level in the hippocampus to inhibit NADPH-d in adult Swiss albino mice exposed to resistant stress.[49] Another study performed on adult Swiss albino mice induced oxidative stress *via* ROS generation, reducing the antioxidant cell defence by depleting glutathione when exposed to lead nitrite. The administration of hydroalcoholic root extract of *Withania somnifera* L. at doses 200 mg/kg/ day and 500 mg/kg/day for 6 weeks increased the brain antioxidant enzymes like superoxide dismutase, catalase, glutathione s-transferase by scavenging ROS, which contributes to the protective effect of *Withania somnifera* L..[50] The neuroprotective potential of *Withania somnifera* L. has also been proved by exposing C6 cells to lead nitrite by balancing glial fibrillary acidic protein (GFAP) expression, heat shock protein (HSP70), and neural cell adhesion molecule (NCAM) expression, the unbalance of the same results in neurodegeneration treated neuronal and glial cell lines IMR32 and C6 with scopolamine, which resulted in the downregulation of brain-derived neurotrophic factor (BDNF) and glial fibrillary acidic protein (GFAP).[51,52] Both are significant for neuronal and glial cells normal growth and upregulation of oxidative stress ROS markers. Withanone upregulated the production of the former and downregulation of the latter. Similarly, Swiss albino mice treated with *Withania somnifera* L. leaf extract at a dosage of 100–300 mg/kg/day exhibited an increase in brain-derived neurotrophic factor (BDNF) and glial fibrillary acidic protein (GFAP), both of which are downregulated when treated with scopolamine alone, indicating brain injury. The neuroprotective effect of the aqueous leaf extract of *Withania somnifera* L. using rat glioma (C6) and human neuroblastoma (IMR0- 32) cell lines was evaluated.[53] The MTT assay was used to assess cell viability and immunocytofluoroscence and Western blot techniques were used to check levels of HSP70. The results demonstrated that the extract protected the retinoic acid, differentiated rat glioma and human neuroblastoma cells against glutamate-induced toxicity characterized by neuronal cell death and an increase in stress protein HSP70. Exposed male Sprague Dawley rats to hypoxia at high altitudes (25,000 ft), which resulted in hippocampal neurodegeneration owing to the formation of free radicals due to low oxygen levels and weakened the antioxidant enzyme system.[22] Administration of Withanolide A at a dosage of 10 µmol/kg before 21 days of pre-exposure and during 7 days of exposure to hypoxia increased glutathione levels in neuronal cells

by up-regulating enzymes for glutathione biosynthesis and gamma-glutamyl cysteinyl ligase *via* the Nrf2 pathway in a corticosterone dependent manner.

Anti-epileptic activity

Withania somnifera L. and its bioactive constituents such as withanolides investigated *via* various *in-vitro* and *in-vivo* models proved to be effective in reducing epileptic seizures through many mechanisms. [54] *Withania somnifera* L. extracts and withanolides have been shown to increase the seizure threshold in the brain in pentylenetetrazol-induced seizures with co-administration of a sub-protective dose of GABA or diazepam and the mechanism involved was GABAergic modulation.[27] Oral administration of *Withania somnifera* L. root extract and withanolide-A at doses of 100 mg/kg/day and 10 mol/kg/day for 15 days restored spatial memory deficit in male Wistar rats with epilepsy induced by pilocarpine *via* inhibiting oxidative stress-induced alteration in glutamergic transmission in the brain by reducing N-methyl-D-aspartate (NMDA) receptor expression. Furthermore, in a pilocarpine- induced epilepsy model, suppressing the -Amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid receptor (AMPA receptor) expression improved motor learning. [55] Similarly, *Withania somnifera* L. root extract and Withanolide-A for 15 days in male was witnessed in Wistar rats at dosages of 100 mg/kg/day and 10 mol/kg/day for inhibiting elevated muscarinic receptor activation in temporal lobe epilepsy, which causes oxidative stress and disrupted cell signalling.[54] The treatment significantly restored the impaired muscarinic receptor activation and oxidative stress and regulated cellular signalling, resulting in a lower prevalence of seizures as compared to the control group.

Anti-Alzheimer activity

Various extracts of *Withania somnifera* L. and its active principles have shown promising results in the treatment of Alzheimer's diseases in many *in-vivo* and *in-vitro* studies by altering different pathological processes like accumulation of amyloid beta plaques in the brain, increased muscarinic receptor binding affinity, etc.[56,57,58,59] The root extract has been shown to block Aβ production, decreasing apoptotic cell death of neurons through the migration of nuclear factor erythroid 2-related factor 2 (Nrf2) to the nucleus.[60] It is a transcription factor that regulates the activity of antioxidant enzymes and protects the cells against oxidative damage.[61] The transcription factor increases the expression of the neuroprotective enzyme heme oxygenase-1.[57,60] Withaferin- A has been shown to inhibit the heat shock protein 90 (Hsp90) and induce heat shock protein 70 & 27 (Hsp27 & Hsp27), whereas the former causes aggregation of τ protein, which is the hallmark of Alzheimer's disease, the latter has a protective role because these inhibit the oligomerization of amyloid-beta in mouse and *drosophila* larval model of Alzheimer's disease. [62 , 63] The thioflavin T fluorescence assay revealed that withaferin-A reduced the production of Aβ, possibly by increasing *a*-secretase expression and decreasing *ß*-secretase expression. [64 , 65] It also increased insulin-degrading enzyme (IDE) production, which causes the degradation of Aβ. In an *invitro* study, it has been found to inhibit AChE obtained from to enhance choline acetyltransferase (ChAT) levels in rats.[66] These two actions of withaferin-A could lead to increased cholinergic transmission in certain areas of the brain, like the basal ganglia and cerebral cortex, which can lead to improved cognitive function by enhancing the binding of acetylcholine to muscarinic M1 receptor. Also, it has been reported that there was no effect on GABAA, NMDA, glutamate and benzodiazepine receptors limiting the side effects.[59] In Alzheimer's disease, the NF-κB pathway blocks the phagocytosis of Aβ fibrils, which leads to the accumulation of Aβ fibrils and neuroinflammation in the brain. [67] Withaferin-A has been shown to inhibit the activation of NF-κB by stopping phosphorylation of NF-κB by inhibiting stimulation of IκB kinase. It also inhibited the activation of NF-κB by attacking the catalytic site of IκB kinase and preventing neuroinflammation.[68]

Hepatoprotective activity

Several studies [69 , 70 , 71] have shown the hepatoprotective potential of *Withania somnifera* L. and its bioactive constituents. Withaferin-A has been reported to diminish the D-galactosamine/lipopolysaccharide-induced acute liver failure in wild type mice by inhibiting the activation of macrophages. The compound improved GalN/LPS-induced hepatotoxicity by targeting macrophage partially dependent on NLRP3 antagonism, while largely independent of Nrf2 signaling, autophagy induction and hepatic AMPKα1 and IκκB studied hepatoprotective activity of aqueous root extract of *Withania somnifera* L. at a dose of 500 mg/kg for 28 days against paracetamol (500 mg/kg) induced hepatotoxcity in female Swiss albino mice. [72,73] The study revealed significant improvement in liver marker enzymes such as AST, ALP, ALT, bilirubin and increased GSH levels compared to control (0.9% NaCl). In another study, Withaferin-A has shown to protect the liver against bromobenzene-induced liver injury in mice by increasing the levels of mitochondrial enzymes, which act as antioxidants by balancing the expression of Bax/Bcl-2 in the liver.[74,75] It has also been shown to inhibit the EMT (epithelial-mesenchymal transition) process, which plays a central role in liver fibrosis by inhibiting the expression of some enzymes like metallopeptidase inhibitor 1 (TIMP1), lysyl oxidase homolog 2

(LOXL2), matrix metalloproteinase-2 (MMP2) which enhances the expression of cadherin-1 (CDH1) leading to the reversal of EMT.[76] Both withaferin-A and withanone decreased the synthesis of inflammatory cytokines *viz.*, TNFa and IL6 in LPS-induced bone-derived macrophages. [77] Further, Withaferin-A inhibited the mitogen-activated protein kinases, including ERK, JNK and NFkB activation, whereas withanone only regulated the ERK and JNK signalling pathways. All these kinases and pathways play a significant role in systemic inflammation, including liver.[72]

Cardioprotective activity

Withania somnifera L. has cardioprotective and cardiotonic properties and is used traditionally for cardiovascular diseases.[78] Various studies on the plant and its bioactive compounds using animal models have proved cardioprotective and cardiotonic effects.[15] Both *Withania somnifera* L. extract and withaferin-A have shown cardioprotective effects in Wistar rats and mice at doses of 40 mg/kg and 1 mg/kg, respectively. In LAD coronary ligation method and reperfusion-induced myocardial injury by decreasing the apoptotic cell death by up-regulating BCl-2 (anti-apoptotic protein) and down-regulating the Bax (pro-apoptotic protein) and thereby reducing the infarct size in the myocardium. [79] A study by on cultured HL-1 cardiomyocytes using an herbal formulation containing *Withania somnifera* L. (0–100 µg/mL) reduced the oxidative damage caused by doxorubicin to these cells by activating transcription factor Nrf-2, which stimulates phase-2 detoxification enzymes which act as antioxidants by scavenging free radicals. 80 Withaferin-A showed anti-platelet and profibrinolytic effects in post-myocardial infarction in normal human plasma by measuring activated partial thromboplastin-time (aPTT) and prothrombin time (PT). Also, TNF-α stimulated human umbilical vein endothelial cells (HUVECs) and inhibited the synthesis of plasminogen activator inhibitor type 1 (PAI-1) with no direct effect on tissue plasminogen activator (tPA), thereby reducing the PAI-1/t-PA ratio which can exert the fibrinolytic effects. [15,28] Evaluated the cardioprotective potential of hydro-alcoholic extract of *Withania somnifera* L. at doses of 25, 50 and 100 mg/kg for 28 days in Wistar albino male rats.[81] A significant decrease in glutathione ($p < 0.05$), activities of superoxide dismutase, catalase, creatinine phosphokinase, lactate dehydrogenase ($p < 0.01$) and an increase in lipid peroxidation marker malonyldialdehyde level ($p < 0.01$) was observed in the hearts of isoproterenol control group rats, the histopathological analysis showed myocardial damage. The increased endogenous antioxidants, maintenance of the myocardial antioxidant status, and significant restoration of altered haemodynamic parameters may contribute to its cardioprotective activity. Evaluated cardioprotective potential at two different doses of withaferin-A in wild-type and AMP-activated protein kinase domain negative (AMPK-DN) transgenic mice.[82] A low dose of 1 mg/kg exerted a cardioprotective effect *via* up-regulating the AMP-activated protein kinase level and decreased the activation of caspase 9 in wild mice, which led to improved cardiac function and reduced infarct size by inhibiting the apoptotic effect of caspase 9, whereas, this protective effect was absent in transgenic mice.

Cognition enhancing activity

Several pre-clinical and clinical studies[83,84,85] have shown that *Withania somnifera* L. enhances the cognitive function in individuals with neurodegenerative disorders, anxiety induced cognitive dysfunction, etc. A dosage of 100 mg/kg/day of *Withania somnifera* L. root extract indicated protection against Propoxur- induced memory loss and reductions in brain and blood cholinesterase activity in male Wistar rats. [86] In a study involving Swiss albino mice, 21-day oral treatment of *Withania somnifera* L. root extract at a dose of 100 mg/kg/day was able to protect the brain against cognitive dysfunction induced by bisphenol and exerted this effect *via* direct scavenging of ROS and by modulating the activity of antioxidant enzymes like catalase and SOD. It was also able to upregulate the NMDA receptor activity, which was downregulated by the bisphenol, the downregulation of the NMDA receptor plays a crucial role in cognitive impairment.[87]

Antiviral activity against SARS-CoV-2

Various *in-silico* studies have shown that bioactive compounds present in *Withania somnifera* L. target a few enzymes and the main spike protein of the virus *via* which it attaches with the host ACE2 receptor and enters the cell.[88] Withanoside and Somniferine have been shown by molecular docking and dynamic stimulation to exhibit a high binding affinity for SARS-CoV-2 main protease enzyme (Mpro) with high binding affinity and have therapeutic potential against COVID-19. [89] Withanoside-V and Withanoside-X by docking studies showed that they possess a strong binding affinity for viral S-glycoprotein which is responsible for the attachment to human ACE2 receptor, highlighting further therapeutic potential against the disease.[90] However, more elaborate *in-vitro* and *in-vivo* studies are needed to investigate the extracts and bioactive compounds of *Withania somnifera* L. and their mode of action against the SARS-CoV-2 virus.

Pharmacokinetics of *Withania somnifera* L.

Different studies have reported the pharmacokinetic

parameters of *Withania somnifera* L. root extracts and the bioactive compounds present in them. It was evaluated that the pharmacokinetic parameters of Withaferin-A in female BALB/c mice using a validated LC-MS/MS method by giving a single dose of Withaferin-A orally to one set of mice and i. v to another group. Maximum plasma concentration (Cmax) was found to be 3,996.90 ± 557.6 ng/mL i. v. and 141.7 ± 16.8 ng/mL orally.[91] Tmax (Time taken to achieve maximum plasma concentration was found to be 0.5 h when the dose was given orally). The oral bioavailability was found to be 1.8%. *In-silico* studies suggested that the low bioavailability may be due to the extensive first pass metabolism by liver enzymes.[92] A study was carried out on the pharmacokinetic parameters of Withaferin-A and Withanolide A and evaluated the oral administration of an aqueous extract of *Withania somnifera L.* at a dose of 1,000 mg/kg of body weight to Swiss albino mice *via* HPLC-MS/MS method. Maximum plasma concentration of Withaferin-A and Withanolide A was found to be 16.69 ± 4.02 ng/mL and 26.59 ± 4.47 ng/mL at Tmax of 10 and 20 min, respectively, suggesting rapid absorption of these bioactive compounds.[93]

Clinical trials of *Withania somnifera* L.

With its widespread use all over the world, it has become necessary to scientifically evaluate all the claimed uses of the root extracts of the herb and other formulations used in the traditional systems of medicine. Many clinical trials have been done to evaluate the efficacy of the herb in relation to human consumption.[94] In a randomized, double-blind controlled study standardized root extract of herb in a dose of 1 gm/day for 12 weeks showed a reduction in positive and negative symptoms in schizophrenia as compared to a placebo.[95] In another study anxiety and depression associated with schizophrenia were improved with 1 gm/day of standardized extract of the root compared to a placebo in the 12-week study on 66 patients.[96] The aqueous root extract of 600 mg/day for 8 weeks in a double-blind randomized controlled trial in a hospital setting resulted in normalization of TSH, T3 and T4 in sub-clinical thyroid patients against a placebo.[97] In a triple-blind randomized control trial in 100 patients with idiopathic infertility and oligospermia administration of capsules containing dried root powder of the herb 5 gm/day was given to one group of patients and the other group received pentoxifylline 800 mg/day for 90 days, both groups showed a significant increase in sperm count and motility and no adverse effect was reported except nausea and epigastric pain in 1 patient, so *Withania somnifera* L. can be an alternative to pentoxifylline in treating male infertility with good safety profile.[98] In a randomized double-blind controlled study involving 66 patients with diabetes mellitus already on metformin therapy received either 250 mg or 500 mg of the aqueous root extract or placebo as an add-on therapy to metformin for 12 weeks. *Withania somnifera* L. group showed significant improvement in HbA1c levels, total cholesterol, triglycerides and LDL, biomarkers of oxidative stress and systemic inflammation were also improved compared to a placebo.[99] In another clinical trial, the efficacy of *Withania somnifera* L. root extract in chronic stress in 64 patients was done against a placebo for a period of 60 days. In this double-blind randomized controlled placebo trial standardized root extract was given in a dose of 300 mg twice daily in the form of a capsule to one group of patients and a placebo was given to another group. The herb-treated group showed a significant reduction in perceived stress score of 44% reduction as compared to only 5.5% in the placebo group, serum cortisol levels were also reduced as compared to the placebo.[100]

Conclusion

The bioactive compounds present in *Withania somnifera* L., such as withanolides possess potential therapeutic activity against many disorders of CNS, cardio vascular system (CVS), inflammatory disorders and liver diseases as they target various biomolecules in living systems which play a major role in these disorders and have proven benefit in animal models of disease. Withanolides such as withaferin-A target multiple pathways of inflammation, cancer, neurodegenerative disorders, etc. However, more clinical trials need to be performed against different forms of cancer and other copious health conditions. The low incidence of side effects of *Withania somnifera* L. makes it the safer choice for its use against various human ailments, but further studies on human subjects are necessary. The proven safety profile of this plant species makes the bioactive compounds potential candidates for clinical trials.

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