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Original Article

A Review of the Sedative, Anti-anxiety and Immunosti-mulant Properties of *Withania somnifera* (L.) Dunal (Ashwagandha)

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ABSTRACT

Background: *Withania somnifera* (L.) Dunal, commonly known as ashwagandha, is a small woody shrub whose roots are used traditionally to treat numerous diseases. It is particularly well known for its sedative, sleep-inducing and immunostimulant effects. **Materials and Methods:** We undertook an extensive search of reviews and primary scientific studies to identify therapeutic uses of *W. somnifera* and studies that have reported on the phytochemistry and validation of the traditional uses. This information was used to identify gaps in the current research that require further study. **Results:** *Withania somnifera* root preparations have widespread uses as sedatives and in the treatment of stress-related conditions. Additionally, there is extensive evidence of the use of *W. somnifera* to stimulate the immune system to protect against pathogenic disease. Numerous studies have examined the mechanisms of action, which are summarised herein. **Conclusion:** Despite the widespread usage of *W. somnifera* roots as a sedative and as an immunostimmulant, the mechanisms of action are not completely understood. Substantial further work is required into the therapeutic mechanism(s) of this useful traditional medicine.

Keywords: Ashwagandha, Solonaceae, Ashwaghandq, Ayuverda, immunomodulation, Sedative, GABA.

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INTRODUCTION

Natural medicines have been used by ancient civilizations since before recorded history. Fossil records indicate human use of plants as herbal medicines as long ago as 60,000 years.[1,2] The traditional Indian medicinal systems Ayurveda, Siddha and Unani, as well as traditional Chinese Medicine (TCM), have particularly long and well documented histories of usage. Many traditional medicinal systems are still practiced as the primary health modality in various regions globally. Even in regions where allopathic medicine dominates, traditional medicines are often used as complementary therapies in addition to the allopathic medicines. Furthermore, numerous allopathic medicines are derived from traditional medicines or are semisynthetic analogues of plant compounds, including atropine, colchicine, digoxin, quinine, and morphine, which are derived from *Atropa belladonna* L., *Colchicum autumanle* L., *Digitalis purpurea* L., *Cinchona legeriana* L. and *Papaver somniferum* L. respectively. These plant-derived compounds often possess potent biological activities and drug-like properties due to their unique chemical diversity and thus are promising resources for the discovery and development of effective new therapies.

Ayurveda is a traditional system of medicine that originated on the Indian subcontinent. It is typically based on complex herbal preparations and has specific diagnostic and therapeutic principles.[3] Nowadays, Ayurveda is often integrated into general health applications as a complementary therapy, rather than just as an alternative to allopathic medicine.[4] In Ayurvedic herbal therapy, *Withania somnifera* (L. Dunal) (family Solanaceae; commonly known as Ashwagandha), is used to treat multiple diseases and ailments.[5] Indeed, *W. somnifera* root is considered to be one of the most important herbs in Ayurveda because of its wide range of health benefits. Some of the most notable medicinal uses of *W. somnifera* root are summarised in Table 1.

Withania somnifera root is most commonly used for relieving stress and anxiety.[6] It has also been reported to enhance strength performance and improve glucose metabolism. *Withania somnifera* roots contain phytochemicals that may reduce inflammation, lower blood pressure, and alter immune system function.[7,8] Studies have reported that *W. somnifera* roots have anxioytic, anti-inflammatory, antioxidant, immunoregulatory and antitumor properties.[6] The purpose of this study is to review the literature on the traditional use of *W. somnifera* root as a sedative and immunostimulative therapy.

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Table 1: Therapeutic effects and mechanisms of action of *Withania somnifera* extracts and purified compounds.

Medicinal Use	Plant part and preparation	Proposed mechanism of action	References
Alzheimer's disease		• Antioxidative mechanism.	9
	Root extracts	• Inhibition of acetylcholinesterase activity	
		• Inhibition of butyrylcholinesterase activity	
		• Neurites regeneration	
		• Increases in corticol muscarinic acetylcholine receptor capacity	
Antibacterial effects	Both root and leaf extracts	• Inhibits *Salmonella* spp. growth	10-12
		• Inhibits the growth of a panel of gastrointestinal pathogens	
		• Inhibits the growth of some bacterial triggers of autoimmune diseases	
		• Potentiates the activity ot rifampicin and isoniazid against *Salmonella typhimurium* and *Escherichia coli*	
Anti-inflammatory properties	Root extracts	• Antioxidative mechanism	13-16
		• Inhibits cyclooxygenase activity and lipid peroxidation	
		• Root extracts decrease Hep2 cell viability and block the cell cycle at G1 phase	
	Isolated withanolides	• Suppresses NF-KB activation	17
Anti-hyperlipidemia effects	Root and fruit extracts	• Antioxidative mechanism	18,19
		• Decreases total lipids, cholesterol and triglycerides but increases plasma HDL levels and HMG-CoA reductase activity.	
		• Decreases lipid peroxidation	
Anti-Parkinson's disease	Root extracts	• Antioxidative mechanism	9
		• Inhibition of acetylcholinesterase activity	
		• Inhibition of butyrylcholinesterase activity	
		• Neurites regeneration	
		• Increases in corticol muscarinic acetylcholine receptor capacity	
Antitumor effects	Root extracts	• Antioxidative mechanisms (decreased levels of glutathione, SOD, CAT and GPX, as well as increased free radical scavenging activity).	13,15,20
		• Decreased incidence of skin cancer.	
	Isolated withanolides	• Pronounced antiproliferative effects in breast and colon cancer cells.	14
Anti-venom	Purified compound	• Inhibits hyaluronidase activity.	21,22
Anxiety and depression	Root extracts	• Increases in GABA levels in the brain.	9

Medicinal Use	Plant part and preparation	Proposed mechanism of action	References
Cardiovascular protection	Purified alkaloid components	• Antioxidative mechanism	23,24
		• Hypotensive, bradycardiac and respiratory-stimulant effects	
Cerebral ischemia	Root extracts	• Antioxidative mechanism	9
Drug addiction	Root extracts	• Antioxidative mechanism	9
Epilepsy	Root extracts	• Antioxidative mechanism	9
		• Increases in GABA levels in the brain	
		• Inhibition of butyrylcholinesterase activity	
		• Neurites regeneration	
		• Increases in corticol muscarinic acetylcholine receptor capacity	
Immunomodulation	Root extracts	• Antioxidative mechanism	20,25,26-30
		• Modulates the levels of immunocompetent cells, immune complexes and immunoglobulins	
		• Stimulates lymphocyte proliferation	
		• Reduction in IL-1 and TNF-α production	
		• Inhibits complement system and mitogen induced lymphocyte proliferation	
	Isolated withanolides	• Activation and mobilisation of peritoneal macrophages, phagocytosis and lysozomal enzymes	5
Memory	Isolated withanolides	• Enhances acetylcholinesterase activity and M1-muscarinic-cholinergic receptor binding	31,32
		• Induces neurites growth	
		• Reverses reserpine-induced retention defects	
Nootropic effects	Isolated withanolides	• Enhances acetylcholinesterase activity and M1-muscarinic-cholinergic receptor binding	31,33
		• Induces neurites growth	
Sexual behaviour	Methanolic root extract	• Marked depression of libido	34
		• Testosterone levels unchanged	
		• Hyperprolactinemic activity	
		• Increases in GABA levels in the brain	
Stress	Root extracts	• Antioxidative mechanism	9
		• Increases in GABA levels in the brain	
Tardive dyskinesia	Root extracts	• Antioxidative mechanism	9
		• Increases in GABA levels in the brain	

Plant profile and taxonomy

Withania somnifera (L.) Dunal is a small woody shrub that grows to approximately 150cm tall (Figure 1a). It occurs naturally in northern Africa, the Mediterranean area and in India, but has also been introduced into other regions of the world. The leaves are simple and ovate (Figure 1b), the flowers are inconspicuous and are globose and orange-red when ripe (Figure 1b) or greenish in colour (Figure 1c). The roots are stout and fleshy (Figure 1d) and are the main part of the plant used medicinally.

Classification

Withania somnifera (L.) Dunal is classified as kingdom plantae; clade tracheophytes; order Solanales; Family Solonaceae; genus *Withania*; species *Withania somnifera* (L.) Dunal.

Synonyms

Withania somnifera has also been known as *Physalis somnifera* L., *Withania kansuensis* Kuang and A.M. Lu, *Withania microphysalis* Seuss.

Nomenclature and common names

The species name "somnifera" means sleep inducing in Latin. The common name "ashwaghanda" is derived from the Sanskrit words "ashva" meaning horse and "gandha" meaning smell.

Phytochemical Composition

Numerous biologically active chemical constituents of *W. somnifera* root have been identified, including include alkaloids, steroidal lactones (withanolides, withaferins), saponins, flavonoids, tannin etc.[35] To date, twelve alkaloids, and thirty-five withanolides that have been reported from *W. somnifera* roots.[35] The major bioactive chemical constituents (withanolides) are steroidal lactones containing an ergostane skeleton and are most prevalent in leaves. The withanolides consist of a C_{28} steroidal scaffold with a C_9 side chain, which contains a six-membered lactone ring.[9,35-37] The structure of the withanolides are similar to the structures of the ginsenosides found in *Panax ginseng* C.A.Mey., resulting in *W. somnifera* often being referred to colloquially as Indian ginseng. The structures of several of the common withanolides identified in *W. somnifera* roots are shown in Figure 2.

Sedatives

A sedative is a substance that induces sedation by reducing irritation or excitability. In 1951, Paul Charpentier synthesized chlorpromazine (one of the earliest clinically available sedatives). Chlorpromazine has found uses to calm patients prior to surgery, as well as to calm patients suffering from hypertension or psychological issues including schizophrenia and manic depression.[38] Sedatives are inhibitors of the central nervous system and interact with brain activity to reduce the activity of certain organs or tissues.[39] They produce beneficial relaxing effects by increasing the activity of GABA.

Immunostimulants

Immunostimulants are substances (drug or nutrient) that stimulates the immune system by inducing activation or increasing the activity of any component of the immune system. These substances can be derived from natural resources or from chemical synthesis. Immunostimulants are divided into specific immunostimulants and non-specific immunostimulants. A specific immunostimulant induces the production of specific antibodies in an immune response (e.g. vaccines). The action of non-specific immunostimulants is independent of the specificity of antigens and relies on enhancing the immune response by stimulating nonantigen-specific components of the immune system.[40] Many immunostimulants activate innate immunity and promote the release of endogenous immune mediators to enhance the natural resistance so that the body can successfully cope with pathogenic challenges. By modulating the expression and activity of specific immune system components, the use of immunostimulants allows for non-specific immunotherapy and non-specific immunoprophylaxis.[41]

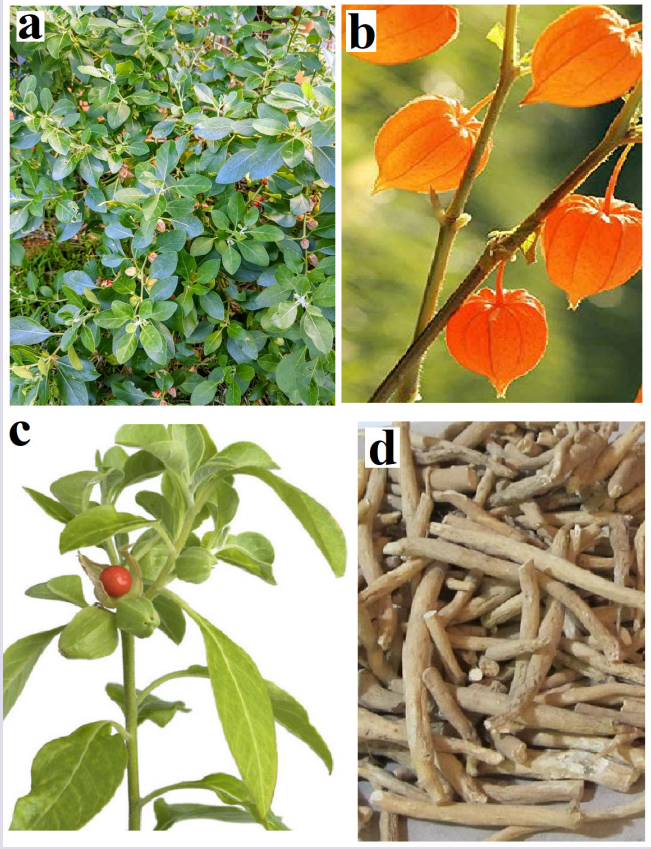

Figure 1: *Withania somnifera* (a) whole plant, (b) seed pods, (c) open seedpod showing the seed and (d) root.

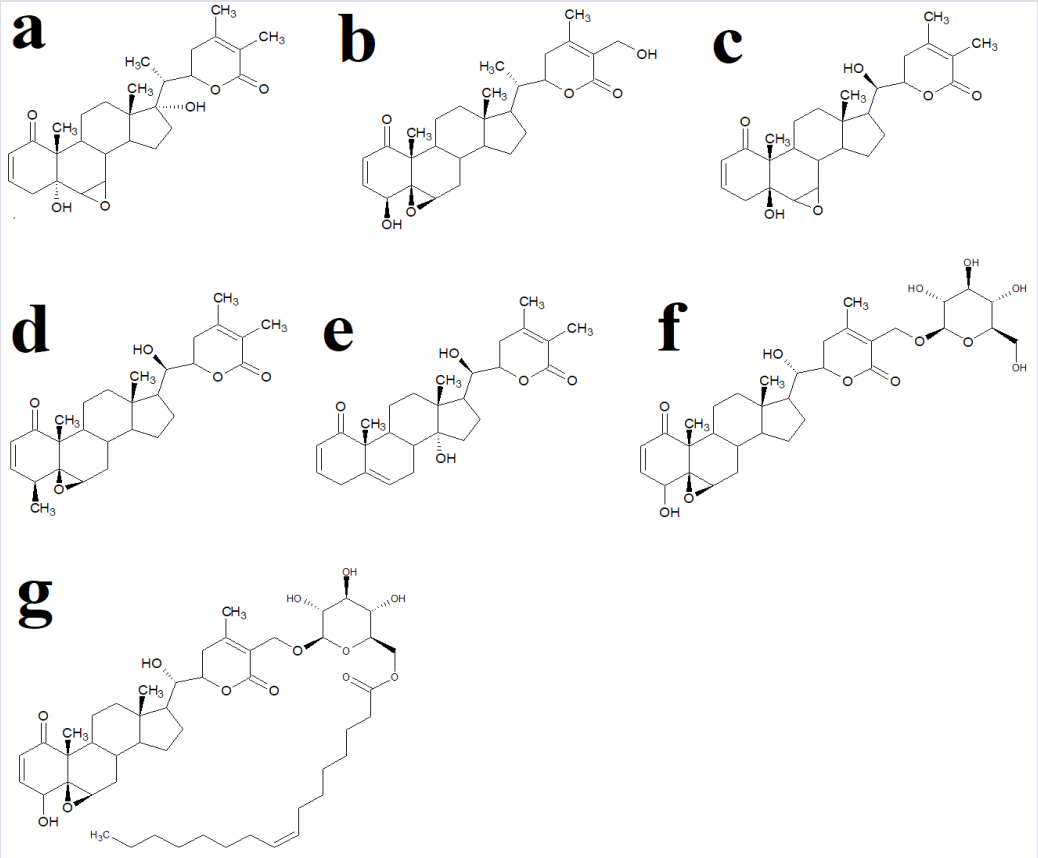

Figure 2: The structures of bioactive withanolide constituents of *W. somnifera*: (a) withanone; (b) withaferin A; (c) withanolide A, (d) withanolide D; (e) withanolide G; (f) sitoindoside IX; (g) sitoindoside X.

The global use of sedatives and immunostimulants

Large doses of sedatives can induce sleep and general anesthesia, whereas lower doses are effective in treating stress, as well as reducing depression and anxiety without interfering with normal brain activity. The most commonly used sedatives include barbiturates and benzodiazepines. Benzodiazepines play an important role in improving sleep patterns, fighting anxiety and alleviating irritability.[42] However, many sedatives have become drugs of abuse due to their psychoactive effects. People who have trouble dealing with stress, anxiety or sleeping often overuse or abuse sedatives, resulting in the development of tolerance to the drugs (which may result in overdoses), requiring increasing doses to maintain the efficacy.[43] Many sedatives are also addictive, and sudden withdrawal after use can produce withdrawal symptoms such as anxiety, sleep disorders, anorexia, nausea, vomiting, hallucinations etc.[44,45] Sedatives may also have serious paradoxical reactions when taken with other drugs, including depression, suicidal tendencies, aggression and violent behaviour.[46] Therefore, these drugs should be used carefully and only under the supervision of medical practitioners.

MATERIALS AND METHODS

The purpose of this study was to review the sedative and immunosuppressant uses and the related biological properties of *W. somnifera* roots in traditional medicine. The information was obtained by searching Google scholar using the following terms as filters, searched both alone and in combinations: "Ashwagandha", "*Withania somnifera*", "withaferin", "sitoindoside", "Indian Ginseng" and "Winter Cherry". The indentified abstracts were obtained and read to determine relevance. Full studies relevant to the sedative and immunostimulant properties of *W. somnifera* roots were subsequently obtained and are reviewed herein.

Eligibility criteria

To meet the eligibility criteria for inclusion in this review, all published studies were required to be English language publications published prior to September 2020. Where possible, the species names were confirmed using the Plant List website (http://www.theplantlist.org/). Where the taxonomic identity of the species could not be definitively verified, the study was omitted from this review.

RESULTS

Sedative properties

Chronic stress can result in multiple adverse effects including alterations of glucose homeostasis, cognitive deficiency, gastric ulcers, immunosuppression, sexual dysfunction and changes in blood hormone levels (e.g. corticosterone). These effects may also have profound downstream effects on an individual's heath. For example, stress-related immunosuppression can increase an individual's susceptible to pathogens, which may result in serious illnesses. Furthermore, individuals suffering from chronic stress tend to also display chronic fatigue.[9] Interestingly, *W. somnifera* root preparations have been reported to decrease the formation of gastric ulcers, return male sexual behaviour to normal and to increase cognition.[6,47] Whilst the aniolytic effects of *W. somnifera* roots are not completely understood, these effects have been linked to the antioxidant capacity of *W. somnifera* preparations. In one study, chronic fatigue induced by stress and excessive activity in mice during a forced swimming experiment resulted in substantial increases in the oxidation of proteins, lipids and DNA.[48] These parameters were significantly reversed in mice provided with *W. somnifera* roots.[48,49]

Similarly, antioxidant activity has been linked with the anti-stress activity of some individual *W. somnifera* components. The antioxidant activity of several glycowithanolides has also been reported in an *in vivo* footshock-induced stress model.[50] Under footshock stress conditions, SOD activity and lipid peroxidation was significantly increased, whilst the enzyme activities of catalase and glutathione peroxidase were substantially decreased. Administration of as little as 10mg of *W. somnifera* extract per kg body weight reversed these stress-related effects. Furthermore, ultrastructural examination of neuronal cells from *W. somnifera* extract treated and untreated animals demonstrated considerable cytoprotection effects, with significant decreases in membrane blebbing and chromatin fragmentation.[51] Further studies reported that the glycowinthanolides sitoindoside VII and sitoindoside VIII,[52] as well sitoindoside IX and sitoindoside X,[53] block a wide range of stress-induced markers in mice and rats.

Anxiety disorders

Withania somnifera has been used for centuries in traditional Ayuverdic practice to alleviate anxiety and stress,[9,54] Interestingly, antagonism of GABA binding to its receptor induces anxiety and stress.[55] Several studies have reported aniolytic effects for *W. somnifera* root extracts and have indicated that the beneficial effects are due to the GABA mimicking properties.[56,57]

Other neurological disorders

Epilepsy

Withania somnifera root extracts have profound anticonvulsant activity in both acute and chronic epilepsy.[9] Indeed, root extracts depress the central nervous system (CNS) and inhibit convulsions in pentylenetetrazol (PZT)-induced kindling *in vivo* in a murine model,[58] as well as amygdaloid kindling[59] and status epilepticus in rats.[60] The effects of PTZ are mediated via interactions with $GABA_A$ gated chloride ionophore and are considered a good model for petit-mal seizures.[9] Therefore, it is likely that a *W. somnifera* compound(s) interfere with this interaction, thereby delaying the onset of the seizures and decreasing their magnitude.

Neurodegenerative diseases

Withania somnifera root and *W. somnifera* containing preparations have been reported to have protective effects against several neurodegenerative disorders. Extracts prepared from *W. somnifera* root reversed 6-hydroxy dopamine-induced Parkinson's-like effects in a rat model at doses between 100 and 300mg extract/kg body weight.[61] That study reported that the extracts reversed multiple oxidative stress parameters including lipid peroxidation, glutathione levels, dopaminergic D_2 dopamine receptor binding, tyrosine hydroxylase expression, as well as glutathione-S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase enzymes activities. Furthermore, a preparation of powdered *W. somnifera* and *Mucuna pruriens* seeds prepared in milk was effective in reducing symptoms of in patients with Parkinson's disease.

Prolonged administration (4 weeks) of *W. somnifera* root extracts also delays the onset and reduces the severity of Tardive dyskinesia (characterised by involuntary repetitive movements of the mouth, face, tongue and sometimes limbs) *in vivo* in a reserpine treatment model.[9,62] The authors of that study also reported that treatment with *W. somnifera* root extract (50-100mg/kg body weight) significantly reduced lipid peroxidation and restored glutathione levels. The same group also reported that the extract also reversed reserpine-induced decreases in brain SOD and catalase levels *in vivo*.[31] Additionally, *W. somnifera* extracts protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced[63] and haloperidol or reserpine-induced catalepsy.[64]

Memory enhancement

Withania somnifera root preparations and isolated compounds have been reported to enhance memory[31,32] and have been proposed as a treatment for Alzheimer's disease.[9,65] Several *W. somnifera* root withanolides inhibit acetylcholinesterase and butylcholinesterase, as well as antagonising calcium.[65] Another study reported that sitoindosides VII-X and withaferin-A (isolated from a methanolic *W. somnifera* root extract) improved cognitive function and decreased amnesia in geriatric patients.[31] Furthermore, isolated withanolide A induced significant neuronal axon and dendrite regeneration in an induced memory deficient mouse model, thereby significantly improving their memory.[66] Additionally, withanoside IV induces neurite outgrowth in cultured rat neuronal cell lines,[66] as well as in SK-N-SH human neuroblastoma cells.[67] Another study reported that withanosides

markedly improved memory in β-amyloid injected mice.[66] That study also reported that isolated withanolides prevented the loss of dendrites, axons and synaptic changes in the hippocampus and cerebral cortex. It has been suggested that *W. somnifera* compounds induce an increase in muscarinic acetylcholine receptors, thereby improving memory and cognition.[31]

Immunostimulant properties of *W. somnifera* roots

The effects of *W. somnifera* roots on the immune system have also been extensively reported. Extracts prepared from *W. somnifera* root enhance white blood cell count in a mouse model and enhanced macrophage phagocytic activity.[26] The extracts induce increased synthesis of nitric oxide synthase, resulting in increased nitric oxide (NO) production in macrophages.[68] Furthermore, several glycowithanolides including sitoindosides IX and X induce macrophage mobilisation and phagocytosis, as well as increasing lysozymal enzyme activity.[5] Another study examined the effects of a *W. somnifera* root extract on macrophage function in mice treated with the carcinogen ochratoxin A (OTA) and reported significant macrophage chemotactic activity.[28] Notably, interleukin-1 (IL-1) and tumour necrosis factor α (TNF-α) production was also significantly decreased following OTA treatment. Another study reported that a *W. somnifera* root extract inhibited NF-Kβ gene expression and NF-Kβ activity, which in turn modulates the activity of multiple genes that control cell proliferation and inflammation.[17]

Additionally, *W. somnifera* roots potently inhibit the complement system, mitogen induced lymphocyte proliferation and delayed hypersensitivity reactions in rats, although they have minimal effect on humoral immune responses.[29] Another study reported that ingestion of aqueous *W. somnifera* root extract in rats immunised with DPT (diptherioa, pertussis, tetnus) vaccine significantly increased antibody titres compared to vaccinated but untreated animals.[30] Similarly, *W. somnifera* root extracts also stimulated immunological activity in Balb/c mice, including significant increases in antibody titres and in the number of plaque forming cells in the spleen, as well as enhanced macrophage phagocytic activity.[27]

DISCUSSION AND CONCLUSION

An extensive literature investigation shows that *W. somnifera* roots are an important source of many important chemical substances. *W. somnifera* root has been successfully used in Ayurvedic medicine for several centuries. It has also been extensively studied for its anti-anxiety, anti-inflammatory, anti-tumour and other pharmacological activities. *Withania somnifera* root plays an important role in as a traditional sedative and immunostimulant in Ayuverda. Other studies have shown that *W. somnifera* roots also have many other pharmacological activities including reducing blood lipid, cardiovascular protection and anti-Parkinson activities. Several studies have verified that the traditional use of *W. somnifera* roots as a medicine and in some cases have provided mechanistic details. However, clinical trials are needed to support the medical use of *W. somnifera* and determine any potential side-effects or cross-reactivities. Additionally, some studies have reported that *W. somnifera* roots have potentiating effects when used in combination with other herbs or drugs and this needs to be explored further.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

- *Withania somnifera* (L.) Dunalroots have been used for centuries in Ayuverdic medicine.
- A literature review was undertaken to summarise the traditional uses of *W. somnifera* roots.
- *Withania somnifera* roots are particularly useful as a sedative, to treat chronic anxiety, and as an immunostimulant.

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