

WITHANIA

Withania somnifera (L.) Dunal

Family

Solanaceae (nightshade family).

This family is comprised of 84 genera that include about 3000 species. Members of this family are generally annual shrubs.¹ The genus *Withania* is closely related to the genus *Physalis*, the gooseberries. *Physalis somnifera* L., *Withania kansuensis* Kuang & A. M. Lu and *Withania microphysalis* Suss. are synonyms.²

Parts Used

Root.

Description

Withania is a small, woody perennial shrub that grows up to one metre in height. It can be found

growing in Africa, the Mediterranean and India. As a result of this wide growing range there are considerable morphological and chemotypical variations in terms of local species. However, the primary alkaloids of both the wild and the cultivated species appear to be the same. It has densely velvety stems and leaves, small white or yellowish flowers in short clusters and spherical orange-red berries of about 8mm in diameter. These are completely enclosed in brown papery and bladderly structures (calyx) formed by the remains of the sepals that enlarge markedly as the fruit develops.^{3,4}

Traditional Use

"One obtains longevity, regains youth, gets a sharp memory and intellect and freedom from diseases, gets a lustrous complexion and strength of a horse." Charaka, Ayurvedic Scholar, 100 BC.



Withania, commonly known as ashwagandha, Indian ginseng or winter cherry (not to be confused with *Physalis alkekengi*, also known as winter cherry), is a revered medicinal plant that has been used in Ayurvedic and indigenous medicine for millennia. Ayurveda, the traditional system of medicine practiced in India, can be traced back to 6000 BC. The genus *Withania* is named after Henry Witham, an English palaeobotanist of the early 19th century.⁵

The species name *somnifera* means sleep-inducing in Latin which probably refers to its extensive use as a remedy against stress from a variety of daily chores. The name ashwagandha is a combination of the Sanskrit word *ashva*, meaning horse and *gandha* meaning smell. The root has been described as having a strong horse-like aroma, although some experts suggest that the origin of the name implies that one who consumes it can attain the power of a horse. While it is considered less stimulating, some herbalists refer to withania as Indian ginseng since it is used in India in a similar way to how *Eleutherococcus senticosus* (Siberian Ginseng) and *Panax ginseng* (Chinese / Korean Ginseng) are used in traditional Chinese medicine to treat a large variety of human diseases. The fleshy roots are indeed superficially similar to ginseng roots. Various parts of withania (berries, leaves and roots) have been used by Ayurvedic practitioners as folk remedies. The berries are sometimes used as a substitute to coagulate milk in cheese making. Classically withania is described as reducing kapha and vata and increasing pitta doshas (body types). It is commonly used in emaciation of children (often given with milk), debility from old age, rheumatism, leucoderma (or vitiligo, a chronic skin disorder that causes de-pigmentation of skin), constipation, insomnia, nervous breakdown and goitre. The paste, formed when roots are crushed with water, is applied to reduce joint inflammation. It is also locally applied in carbuncles, ulcers and painful swellings. The root, in combination with other drugs, is prescribed for scorpion-sting as well as snake venom.

In *in vitro* studies withania glycoprotein inhibits the hyaluronidase activity of cobra (*Naja naja*) and viper (*Daboia russelii*) venoms providing some support for this use.⁶

It also helps in leucorrhoea, boils, pimples, flatulent colic, worms and piles. The leaves are bitter and

are recommended in fever and painful swellings. The flowers are astringent, depurative, diuretic and aphrodisiac. The seeds are anthelmintic. In Ayurveda withania is referred to as a 'rasayana', a group of plant-derived drugs reputed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalise the body in debilitated conditions and increase longevity. These types of remedies are given to small children as tonics and are also taken by the middle-aged and elderly to increase longevity. Among the ayurvedic rasayana herbs, withania holds the most prominent place. Most of the rasayana herbs are adaptogen and antistress agents. Withania is commonly available as a churna, a fine sieved powder that can be mixed with water, ghee (clarified butter) or honey. It enhances the function of the brain and nervous system and improves the memory. It improves the function of the reproductive system promoting a healthy sexual and reproductive balance. Being a powerful adaptogen, it enhances the body's resilience to stress. Withania improves the body's defence against disease by improving the cell-mediated immunity. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals. In view of its varied therapeutic potential, it has also been the subject of considerable modern scientific attention. Withania roots are a constituent in more than 200 formulations in Ayurveda, Siddha and Unani medicine, which are used in the treatment of various physiological disorders.^{7,8,9,10}

Constituents

The chemistry of withania has been extensively studied and more than 80 chemical constituents have been identified, extracted and isolated. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII) and withanolides with a glucose at carbon 27 (sitoindoside IX and X). It is also rich in iron, phytosterols (beta-sitosterol, stigmasterol, beta-sitosterol glucoside, stigmasterol glucoside), triterpene (beta-amyrin), Viscosa lactone B, alpha+beta glucose. Two withanolides, withaferin A and withanolide D, are regarded as quality markers.^{12,13,14,15}

Actions

Adaptogen, anxiolytic, nervine tonic, tonic, mild sedative, aphrodisiac, anti-inflammatory, antitumor, antioxidant, immunomodulatory, cardioprotective.

Pharmacological Activity

Findings from studies on withania clearly indicate that its traditional use has a logical and scientific basis however large scale clinical studies are needed to prove the clinical efficacy of this herb, especially in stress related diseases, neuronal disorders and cancers.¹⁶ Whilst the pharmacological actions of individual components is important to understand clinical effects are difficult to predict from these studies as the ultimate effect of the herbal treatment will be the result of many intraherbal interactions.¹⁷ This monograph will focus on whole herb studies.

Adaptogenic and Antistress Activity

A recent systematic review of five human randomised controlled clinical trials (RCT) with 400 participants found that withania is effective at reducing symptoms of stress and anxiety. All five trials reported at least one significant benefit of the herbal medicine in comparison with control conditions for anxiety and/or stress related outcomes. The doses of the herbal medicine ranged from 125 to 1,200mg per day, with trials being between six to 16 weeks.

However the researchers said the strength of trial results may be very limited by factors of potential bias. Since this review one further eligible study has been conducted. A double-blind RCT in 52 people with chronic stress, comparing 600mg of withania per day to placebo over eight weeks. Results revealed significantly greater reductions in stress outcomes from withania, corresponding with significant decreases in salivary cortisol (a stress/anxiety biomarker).^{18,19}

An open-label prospective non-randomised comparative trial on 100 breast cancer patients, in all stages undergoing either a combination of chemotherapy with oral withania or chemotherapy alone, concluded that withania is useful against cancer-related fatigue in addition to improving the quality of life in breast cancer patients undergoing

chemotherapy. The chemotherapy regimens were either taxotere, adriamycin and cyclophosphamide or 5-fluorouracil, epirubicin and cyclophosphamide. Withania was administered to patients in the study group at a dose of 2g every eight hours throughout the course of chemotherapy. The majority (77%) of patients had stage II and III disease.²⁰

The findings of a recent single centre, prospective, double-blind, randomised, placebo-controlled trial suggest that withania improves an individual's resistance towards stress and improves self-assessed quality of life and therefore can be used safely as an adaptogen in adults who are under stress. A total of 64 subjects with a history of chronic stress were enrolled into the study and took one capsule (300mg of high-concentration full-spectrum withania extract) twice a day for a period of 60 days. The treatment group showed a significant reduction in scores on all the stress-assessment scales on Day 60 and their serum cortisol levels were substantially reduced.²¹

A six week, double-blind, randomised controlled trial was conducted to evaluate the efficacy of an ethanolic extract of withania (500mg twice daily) in 39 patients with a range of anxiety disorders. Twenty patients received the drug and 19 received placebo. At two and six weeks follow-up, data from approximately 85% of patients in each group was available for analysis. Statistical trends favouring withania were observed at both time points.²²

A randomised, controlled trial studying the effectiveness of naturopathic care on anxiety symptoms was conducted comparing the use of withania (300mg twice a day standardised to 1.5% with anolides) in conjunction with dietary counseling, relaxation techniques and multivitamins. Along with a placebo the control group was given psychotherapy and breathing exercises. Seventy-five participants were followed for eight or more weeks on the trial. After at least two months on the protocols patients in both groups were evaluated. Those in the group who had naturopathic care experienced significantly lower levels of anxiety. In addition, the study authors concluded that significant differences between the groups were also observed in mental health, concentration, fatigue, social functioning, vitality, and overall quality of life with the withania group exhibiting greater clinical benefit.²³

Sarcopenia is the age related degenerative loss of muscle mass, strength and endurance. It leads to increased morbidity in elderly people. A recent preliminary clinical trial to study the therapeutic benefit of withania in this disorder was performed by recruiting 35 people aged between 55 and 75 years. The authors concluded that extracts from withania have the potential to improve muscle strength and endurance in aged subjects, and it has a strong role in the management of age related disorders. The participants of the trial were given a 500mg withania capsule twice daily for three months. Muscle strength and muscle functional performance were tested for all participants prior and post-trial. Blood samples were drawn prior and post-trial for creatine kinase testing to provide evidence on changes in muscle metabolisms. The authors said that although no valid statistical test could be conducted for these tests due to the limited sample size, the trend is promising. Despite the limited sample size the authors said they could not ignore the evidence that the blood creatine kinase levels declined a lot in the three month trial. The changes in blood creatine kinase levels with the use of withania suggest a possible increase in muscle metabolism or a possible decline in muscle catabolism.²⁴

There have been extensive studies using animal models demonstrating the adaptogenic and antistress properties of withania. Studies have shown it to be effective in increasing stamina (physical endurance) and preventing stress induced gastric ulcer, induced hepatotoxicity and mortality.

Its antistressor properties were investigated in a study using rats and cold water swimming stress test. The results indicated that the withania treated animals show better stress tolerance.²⁵

An animal study has observed adaptogenic effects of ashwagandha via a stress-inducing procedure revealing attenuation of stress-related parameters including cortisol levels, mental depression and sexual dysfunction.²⁶

A withanolide-free hydrosoluble fraction was isolated from the roots of withania. It exhibited significant antistress activity in a dose-related manner and was further studied against chemical and physical induced stress in rats and mice.²⁷

A methanolic extract of withania root was found to inhibit the specific binding of gamma-aminobutyric

acid (GABA) ligands and enhanced the binding of flunitrazepam (also known as Rohypnol, an intermediate acting benzodiazepine used as a hypnotic, sedative, anticonvulsant, anxiolytic and skeletal muscle relaxant drug) to their receptor sites, displaying GABA-mimetic activity.²⁸

Sedative Activity

The alkaloids in withania are said to be sedative and reduce blood pressure and heart rate.²⁹ An Indian study explored the role of withania root extract in sleep-disturbed rats. The results suggest the involvement of a GABA-ergic mechanism in the sleep promoting effects of withania in a sleep-disturbed state.³⁰ An earlier study by the same authors investigated the protective effect of withania on the behavioural and biochemical alterations in sleep disturbed mice. The results suggested that withania root extract can be used in the management of sleep loss and associated oxidative stress. Pretreatment with withania root extract (100 and 200mg/kg) and diazepam (0.5mg/kg) significantly protected reduction in body weight, improved the reduced locomotor activity and anxiety levels in animals.³¹

Nervous System Activity

Cognitive enhancement

The results of a 2014 prospective, double-blind, multi-dose, placebo-controlled, crossover study suggest that withania can improve cognitive and psychomotor performance and may, therefore, be a valuable adjunct in the treatment of diseases associated with cognitive impairment. Twenty healthy male participants were randomised to receive 250mg two capsules twice daily of an encapsulated dried aqueous extract of roots and leaves of withania or a matching placebo for a period of 14 days and had cognitive tests done before and after the two week regimen. After two weeks they showed significant improvements in cognitive ability.³²

The cognitive effects of a standardised extract of the withania showed promise in bipolar disorder in an eight week, double-blind, placebo-controlled, randomised study. Sixty euthymic (normal mood) subjects with bipolar disorder were enrolled in the study of withania (500mg/d) as a procognitive

agent added adjunctively to the medications being used as maintenance treatment for bipolar disorder. Although results are preliminary, withania appears to improve auditory-verbal working memory, a measure of reaction time, and a measure of social cognition in bipolar disorder. See below in thyroid activity for more information on this study.³³

The results of an *in vitro* study may partially substantiate the traditional use of withania for improvement of cognition. The Indian study showed that a methanolic extract of withania had potent acetylcholinesterase inhibitory activity (an important therapeutic strategy in Alzheimer's disease).³⁴

Withania exhibited a memory enhancing effect in mice in an Indian study. Daily administration of withania for six days significantly improved memory consolidation in mice receiving chronic electroconvulsive shock treatment.³⁵

An earlier German study was conducted to assess whether the memory-enhancing effects of plant extracts from withania are owing to neurochemical alterations of specific transmitter systems. The data suggested withania affects, preferentially, events in the cortical and basal forebrain cholinergic signal transduction cascade.

The authors concluded that the drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition-enhancing and memory-improving effects of extracts from withania observed in animals and humans.³⁶

Neuroprotective

A 2017 study has shown the potential of withania (and *Centella asiatica*) to ameliorate Parkinson's disorder, a neurodegenerative disorder. Similar to previous work on ethanol extracts of these two plants in mice models the researchers saw an improvement in oxidative stress profile as well as behavioral performance in induced Parkinson-like symptoms in mice. Given the known potential of both the herbal extracts in improving Parkinson-like symptoms they expected the combination of the two to show better results than either of the two but surprisingly there was no additivity in either oxidative stress or behavioural recovery. In fact, in some assays, the combination performed worse than either of the two individual constituents.

The researchers said the two most important topics to come out of this project are the need to study synergism and the need for more effective visualisation tools (in the form of complete comparison display) to enable better analysis of results.^{37,38}

A recent study evaluated the neuroprotective effect of withania extract on Parkinsonian mice. The mice were divided into three groups; the first group served as control, the second group was given maneb (MB) and paraquat (PQ) and the last group was administered MB-PQ along with withania for three, six and nine weeks. Maneb is a foliate fungicide used to create a toxin-based animal model of Parkinson's disease and paraquat is a herbicide linked to Parkinson's disease. The behavioural studies showed a significant improvement in the motor movement patterns and gripping ability of withania exposed Parkinsonian mice. The results clearly indicated the usefulness of withania in providing protection against MB-PQ induced nigrostriatal (one of the major dopamine pathways in the brain involved particularly in the production of movement) dopaminergic neurodegeneration and marked improvement in the behavioural, anatomical and biochemical deformities.³⁹

Another animal study found that withania has neuroprotective and prophylactic potential. Administration of withania prevented induced memory impairment and neurodegeneration along with decreased nitric oxide, corticosterone, oxidative stress and acetylcholinesterase activity in the hippocampal region.⁴⁰

In an earlier study the neuroprotective effects of withania were studied on stressed rats. Treatment with withania significantly reduced (80%) the number of degenerating cells in both the areas.⁴¹

Another indication for withania's potential to protect neurons was demonstrated in a study which found the methanol extract of withania promotes the formation of dendrites. Extension of dendrites and axons in neurons may compensate for and repair damaged neuronal circuits in the dementia brain.⁴²

The findings of an Indian study suggest that phytochemicals present in withania mitigate the effects of excitotoxicity (pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters) and

oxidative damage in the hippocampus and this might be accomplished by their antioxidative properties.⁴³

Thyroid Activity

Withania may be useful for hypothyroidism. It has been found to increase serum concentrations of thyroid hormones in animal studies by stimulating thyroid function and a 32-year-old healthy woman developed thyrotoxicosis (hyperthyroidism with symptoms such as weight loss, palpitations, confusion) after taking withania capsules for symptoms of chronic fatigue. She was reportedly not taking any other remedies or medications. During the first few weeks she took the capsules only occasionally without any symptoms but, after increasing the dose, she experienced clinical symptoms indicative of thyrotoxicosis. This was confirmed by laboratory assessment. The symptoms resolved spontaneously after discontinuation of the withania capsules and laboratory values normalised. The reported dose was 500 mg/day of withania, possibly of a dry extract.^{44,45,46}

Despite the above case report a recent study in bipolar patients did not see any adverse impact of withania on normal thyroid function.

Laboratory indices of thyroid function (TSH, Free T4 and T3) were measured in a randomised clinical trial in which withania was used to improve cognitive function in patients with bipolar disorder (See above in cognitive enhancement). This was done in light of the case-report and *in vivo* study above which showed significant increases in thyroxine levels. Dysfunction of the hypothalamic-pituitary-thyroid neuroendocrine axis has been implicated in the pathogenesis of major depressive and bipolar disorders. Moreover, symptoms such as fatigue, tiredness, mental slowing, concentration and memory impairments, weight gain and depression are common to both overt hypothyroidism and to unipolar depressive and bipolar disorders. Taking this into account, and as the relationship between lithium and hypothyroidism is well known, thyroid status is often assessed and monitored in mood disorders. Furthermore, there is some support for adjunctive treatment with thyroxine in treatment-resistant depression and in rapid-cycling bipolar disorder. As thyroid indices were done for safety in this study, and not the primary goal of the original study, only a small fraction of trial participants

exhibited abnormal thyroid readings. Ten (of the original 60) patients showed abnormal results in one of the thyroid measures (TSH, free T4 and T3) either at the beginning or end of the eight week study. Since the parent study was not designed to primarily examine thyroid function in bipolar patients treated with withania the sample size was small and unequally divided between patients who received placebo (n = 7) or withania (n = 3) and the thyroid tests were not repeated for confirmation. One withania-treated patient had subclinical hypothyroidism (TSH 5.7 mIU/L) at baseline that normalised, and all three withania-treated patients experienced T4 increases from baseline (7%, 12%, and 24%). Six of seven placebo-assigned patients showed decreases in T4 from baseline (4% to 23%), and one patient's TSH moved from normal to the subclinical hypothyroid range (6.96mIU/L). The authors concluded that in spite of the limitations, the subtle laboratory changes noted in thyroid indices in this eight week study suggest that withania may increase thyroxine levels, and therefore vigilance regarding hyperthyroidism may be warranted.

Nonetheless, the thyroid-enhancing properties of withania may also represent a clinical opportunity for the treatment of subclinical hypothyroidism. The scientists went on to say that this study highlights the importance of thyroid function testing before starting withania among Ayurvedic or other healthcare practitioners and emphasises that repeat testing may be required if there is clinical suspicion of hyperthyroidism during withania treatment.⁴⁷

Anti-inflammatory Activity

A recent study has found that withania acts as an anti-inflammatory and antioxidant agent in decreasing the arthritic effects in arthritic rats and has a protective effect. The study focused on the effect of withania root powder on the behavioural and radiological changes in arthritic rats. Administration of withania root powder (600mg kg⁻¹) to the arthritic rats significantly decreased the severity of arthritis by effectively suppressing the symptoms of arthritis and improving the functional recovery of motor activity and radiological score.⁴⁸

A recent *in vitro* study has shown direct, statistically significant, anti-inflammatory effects of an aqueous extract of withania on human osteoarthritis

cartilage. Withania significantly decreased nitric oxide release in one subset of patients (potentially decreasing blood flow).⁴⁹

Antiosteoporotic Activity

A recent *in vivo* study examined the effect of an ethanolic withania extract for antiosteoporotic activity. Withania treatment markedly prevented the negative changes in the tested animals and thus the researchers concluded withania may be a potential agent in the treatment of osteoporosis.⁵⁰

Chemotherapeutic Activity

In a 2017 *in vitro* study researchers investigated the potential of withania as a 'priming' agent, and showed that 'priming' (where cancer cells are treated with a priming agent to sensitise cells prior to chemotherapy treatment) with this root extract enhanced the efficacy of cisplatin (a chemotherapy drug) through increased reactive oxygen species (ROS) in cancer cells while having no detectable effect on non-cancer cells. The underlying mechanism underpinning 'priming' appears to be the enhancement of cell death through mitochondrial dysfunction. It has been suggested that mitochondria are the gatekeepers to chemotherapy and are ideal therapeutic targets for cancer therapy, therefore the impact of withania in enhancing chemotherapy through mitochondrial dysfunction may prove an important new approach. Withania has been found to be effective in treating several types of cancer including skin, leukaemia, breast, colon and pancreas. The mechanisms of action have yet to be fully elucidated but indications of involvement in mitochondrial membrane permeability have been reported in several *in vitro* studies. Additionally withania has been shown to increase ROS. The mitochondria is an important regulator of cell survival and progression and is the main source of ROS which is linked with mitochondrial function. Cancer cell metabolism is known to have an altered phenotype whereby they primarily respire through lactate production in a process known as the 'Warburg Effect'. This alteration in metabolism is a key hallmark of cancer cell progression and has been linked to an alteration in mitochondria function. Withania has been reported to induce mitochondrial dysfunction in human leukaemia cells and also reduce mitochondrial function in breast cancer cells.

Mitochondrial dysfunction can alter ROS levels, ATP production and overall cell viability and is a novel key target in cancer treatment.⁵¹

The combination of paclitaxel (a drug used in cancer chemotherapy that inhibits cell division) with withania could effectively treat induced lung cancer in mice by offering protection from reactive oxygen species damage and also by suppressing cell proliferation. The data suggests that paclitaxel, administered with withania, may extend its chemotherapeutic effect through modulating protein-bound carbohydrate levels and marker enzymes, as they are indicators of cancer.⁵²

A protective effect in induced myelosuppression was observed in animals treated with withania, revealing a significant increase in white blood cell counts and platelet counts.⁵³

Antitumour Activity

A recent *in vivo* study has found withania may enhance antitumour function of natural killer which play a major role in the host-rejection of both tumours and virally infected cells.

This study may be useful for a clinical study to determine the effects of dietary withania on NK cell immune function in patients with ovarian cancer. The results of this study suggest that the population of stromal and tumour-infiltrating NK cells is increased by dietary withania supplementation which may indicate an ongoing immune response toward the tumor.⁵⁴

A preclinical study has found that withania may be useful in the management of malignancy by targeting multiple pathways. The results of the study demonstrate that withania exhibited selective cytotoxicity against a panel of human cancer cell lines *in vitro* compared to normal cells and effectively inhibited tumour growth in mouse tumour models. Besides its antitumour effect, withania also stimulated the cell-mediated Th1 immune response in tumour-bearing mice.⁵⁵

The Chinese Hamster ovary (CHO) cell line is widely used for measuring drug cytotoxicity and resistance. Withania caused a reproducible, dose dependent, inhibition of colony formation of CHO cells. The authors said this knowledge will assist oncologists who plan to use withania extracts as 'synergisers'

with conventional chemotherapy or radiation therapy.⁵⁶

An article reviewing the literature pertaining to withania and its botanical constituents as antitumour agents, in conjunction with radiation and chemotherapy treatment, concluded that it reduces tumour cell proliferation while increasing overall animal survival time. Withania was also shown to enhance the effectiveness of radiation therapy while potentially mitigating undesirable side effects. It also reduces the side effects of chemotherapeutic agents cyclophosphamide and paclitaxel without interfering with the tumour-reducing actions of the drugs. These effects have been demonstrated *in vitro* on human cancer cell lines, and *in vivo* on animal subjects. Given its broad spectrum of cytotoxic and tumour-sensitising actions, the author concluded that withania presents itself as a novel complementary therapy for integrative oncology care.⁵⁷

Immune and Blood Building Activity

One small study showed patients taking 6mL of withania twice a day showed a major change in immune cell activation in 96 hours. Five participants consumed 6mL of a withania and whole milk twice daily for 96 hours.

At 96 hours of use mean values of receptor expression for all measured receptor types were increased over baseline, indicating that a major change in immune cell activation occurred across the sample.⁵⁸

A study investigating chronic stress-induced alterations on Th1 lymphocyte subset distribution and corresponding cytokine secretion patterns in mice has found withania causes a significant increase in the stress-induced depleted T-cell population and increases the expression of Th1 cytokines in chronically stressed mice.⁵⁹

Improved haematopoiesis⁶⁰ and significant increases in haemoglobin concentration, red blood cell count, white blood cell count and platelet count have been observed *in vivo*. The immunomodulatory activity of withania was studied in mice with myelosuppression induced by one or more of the following three compounds: cyclophosphamide, azathioprin or prednisolone. A significant modulation of immune reactivity was observed in all the three animal models used. Withania prevented myelosuppression

in mice treated with all three immunosuppressive drugs tested. A significant increase in haemoglobin concentration, red blood cell count, white blood cell count, platelet count and body weight was observed in withania-treated mice as compared with untreated (control) mice. The authors also reported an immunostimulatory activity: treatment with withania was accompanied by significant increases in haemolytic antibody responses towards human erythrocytes.⁶¹

Antioxidant Activity

Reported antioxidant properties include prevention of lipid peroxidation in rats. A study was carried out to evaluate the antiperoxidative effect of withania on liver lipid peroxidation and antioxidant status in adjuvant induced arthritic rats. Results were compared with those for Indomethacin, a non-steroidal anti-inflammatory drug. The oral administration of withania (1000mg kg⁻¹ b.wt.) modulated the above altered lipid peroxidation and antioxidant status to near normal levels in arthritic animals.⁶²

Reproductive Activity

Withania has been shown to have a positive effect in the treatment of infertility in both males and female. The mechanism of withania's effect on the reproductive system is not entirely known yet but it is thought to be linked to its antioxidant features and ability to improve the hormonal balance of luteinizing hormone, follicle stimulating hormone and testosterone and improve detoxification processes. A GABA mimetic action is thought to play the main role in inducing gonadotropin releasing hormone secretion and improving hormonal balance. In the male reproductive system it is assumed that by providing metal ions withania facilitates enzyme activities, modifies oxidative stress and prevents cell apoptosis. Withania has been shown in human trials to induce alanine transaminase activity which increases alanine in seminal fluid leading to a less oxidative stress index and improved semen quality. By normalizing lactate, phenylalanine, glutamine, citrate and histidine in seminal fluid withania improves enzymatic processes.⁶³

In a 2018 triple-blind randomised clinical trial researchers found that withania improves sperms parameters in unexplained male infertility without

causing adverse effects. The effects of withania and pentoxifylline (a medication used to improve the symptoms of a blood flow problem in the legs/ arms but also used to increase sperm motility) on the sperm parameters in unexplained male infertility were compared. One hundred infertile male patients were randomly allocated into either withania or pentoxifylline groups. Patients in the withania group received six capsules containing 5g/daily of withania, and subjects in the pentoxifylline group received six capsules containing 800mg/daily of pentoxifylline and placebo for 90 days. Sperm parameters were analysed at the beginning and end of the study. Withania increased mean sperm count and progressive motility and improved sperm morphology.⁶⁴

A 2018 systemic review on the therapeutic effects of withania on the reproductive system found that it enhanced spermatogenesis and sperm related indices in males and sexual behaviors in female. There were 42 studies included. Of these eight were human (seven studies on men and one on women – see below for summaries), 28 animal studies (20 on males and eight on females), five animal-cellular studies and one cellular study.

Of these studies 29 were done on the roots and all human studies used the roots. The duration of human studies ranged from 60 to 90 days. Withania was found to improve reproductive system function in many ways. It decreased infertility among male subjects due to the enhancement in semen quality which is suggested to be due to the enhanced enzymatic activity in seminal plasma and decreasing oxidative stress. It also improved luteinizing hormone and follicular stimulating hormone balance leading to folliculogenesis and increased gonadal weight, although it should be noted that some animal studies had concluded that withania had reversible spermicidal and infertiling effects in male subjects.⁶⁵

In a recent study about the effects of withania on men it was shown that after using it for three months sperm parameters such as count and motility in sperm analysis had improved due to decreased apoptosis and reactive oxidative stress among men with normospermia and oligospermia. Also the essential metal ions of seminal plasma, copper, zinc, iron and gold, had increased after the treatment and subsequently semen quality

increased. The researchers postulated that this increase in semen quality was due to the increase in essential neurotransmitters, metallothionein, which has antioxidative functions, and metal ions as cofactors for essential enzymes.⁶⁶

A human clinical trial found that treatment with withania (5g orally per day for three months with a cup of skimmed milk) resulted in a decrease in stress and a significant decrease in cortisol levels in a group of men with stress-induced infertility. Stress has been reported to be a causative factor for male infertility. This trial was conducted to understand the role of stress in male infertility and to test the ability of withania to combat stress and treat male infertility (see also spermatogenic activity below). A total of 121 men were selected for the study. Normozoospermic (normal semen) but infertile individuals were chosen and further categorised in three groups: normozoospermic heavy smokers, normozoospermics under psychological stress and normozoospermics with infertility of unknown etiology. Normozoospermic fertile men were recruited as controls.

Measuring various biochemical and stress parameters before and after treatment suggested a definite role of stress in male infertility and the ability of withania to treat stress-related infertility. Treatment resulted in a decrease in stress, improved the level of antioxidants and improved overall semen quality in a significant number of individuals. The treatment resulted in pregnancy in the partners of 14% of the patients. The authors found that among withania's major effects it balances hormone levels, reduces oxidative stress and possibly improves detoxification processes in the body. In an earlier study it was shown that disturbed hormone levels correlate well with infertility therefore correction of this imbalance by withania could be one of the major factors contributing to fertility improvement. Interestingly, the authors stated that it could be a combination of direct and indirect effects of this herb to combat stress by pleuripotent (the ability of certain substances to produce several distinct biological responses) effector constituents. They said "It would be worthwhile to stress that such an effect is more likely in its natural mixture form than isolating one or more individual fractions that may not yield similar effects. However, it is still a

good idea to fractionate this herb to understand its mechanism of action.”⁶⁷

A prospective study showed that withania improved semen quality in infertile males by regulating reproductive hormone levels and seminal plasma oxidative stress. The study included 75 normal healthy fertile men (control subjects) and 75 men undergoing infertility screening.⁶⁸

It has been well documented that high levels of reactive oxygen species (ROS) in the semen induce oxidative damage to the sperm and are associated with abnormal sperm parameters leading to infertility. Withania has been found to counteract the formation of ROS in infertile men.^{69,70,71,72,73}

A recent study indicated that treatment with a high-concentration, full-spectrum root extract of withania results in significantly improved semen parameters together with improved, and regulated, sexual hormone levels in oligospermic (low sperm concentration) males. The analyses of the data indicated significantly increased sperm concentration and overall motility, which are regarded as the most important criteria for normal fertilising ability of the spermatozoa.

The study outcome showed significant enhancement of the semen volume in the withania-treated infertile males. Forty-six male patients with oligospermia (sperm count < 20 million/mL semen) were enrolled and randomised either to treatment (n = 21) with a full-spectrum root extract of withania (675 mg/d in three doses for 90 days) or to placebo (n = 25) in the same protocol. Semen parameters and serum hormone levels were estimated at the end of 90-day treatment. There was a 167% increase in sperm count, 53% increase in semen volume and 57% increase in sperm motility on day 90 from baseline. The improvement in these parameters was minimal in the placebo-treated group. A significantly greater improvement and regulation was observed in serum hormone levels with the withania treatment as compared to the placebo. In the study, treatment with the withania root extract resulted in a higher level of testosterone and a concomitant increase in serum levels of luteinizing hormone among infertile men having suboptimal testosterone levels before therapy. Apart from spermatogenesis, testosterone also controls the functional competence of the accessory sex

organs, as adequate seminal fluid is necessary for the survival and motility of spermatozoa. Thus, it was postulated that the probable reasons for the increased sperm concentration and motility in the findings lie in the higher levels of testosterone. These observations have been reported by other workers investigating the fertility-enhancement potential of withania and other herbs and minerals.⁷⁴

Experimental studies have shown that treatment with withania induced testicular development and spermatogenesis *in vivo* by directly affecting the seminiferous tubules, improved prosexual behaviour of sexually sluggish mice and increased testicular daily sperm production and serum testosterone level.^{75,76,77}

Antibacterial and Antifungal Activity

A study on the antibacterial activity of withania has found that oral administration of the aqueous extract of withania successfully obliterated salmonella infection in mice as revealed by increased survival rate, as well as less bacterial load in various vital organs of the treated animals. Both aqueous as well as alcoholic extracts of the plant (root as well as leaves) were found to possess strong antibacterial activity against a range of bacteria *in vitro*.

The methanolic extract was further subfractionated using various solvents and the butanolic sub-fraction was found to possess maximum inhibitory activity against a spectrum of bacteria including *Salmonella typhimurium*. In contrast to the synthetic antibiotic (chloramphenicol), these extracts did not induce lysis (breaking down of the cell wall) on incubation with human erythrocytes, advocating their safety to the living cells.⁷⁸

The methanol and hexane extracts of both leaves and roots of withania were found to have potent antibacterial activity when evaluated *in vitro* for the antibacterial/synergistic activity against *Salmonella typhimurium* and *Escherichia coli*.⁷⁹

Cardiovascular Activity

Withania improved the cardiorespiratory endurance of elite athletes in a recent study. Forty elite Indian cyclists were chosen randomly and were equally divided into experimental and placebo groups. The experimental group received 500mg capsules of withania twice daily for eight weeks, whereas the

placebo group received starch capsules. There was significant improvement in the experimental group in all parameters, whereas the placebo group did not show any change with respect to their baseline parameters.⁸⁰

In a 2018 *in vivo* study withania showed protective effects in ischemic stroke. Pre-supplementation with withania ameliorated oxidative stress and mitochondrial dysfunctions in the experimental animal model of stroke.⁸¹

The antioxidant and antiapoptotic properties of withania may contribute to its cardioprotective effects. An animal study was undertaken to evaluate the cardioprotective mechanisms of withania, in the setting of ischemia (decreased blood flow and oxygen to the heart muscle) and reperfusion injury (the tissue damage caused when blood supply returns to the tissue after a period of ischemia). Rats receiving prior treatment with withania favourably restored the myocardial oxidant-antioxidant balance, exerted marked antiapoptotic effects and reduced myocardial damage.⁸²

Hypoglycaemic Activity

Withania has been studied for its potential benefits for those with type 2 diabetes with one small study showing a decrease in blood glucose comparable to an oral hypoglycemic drug, as well as significantly decreased low density lipoproteins and very low density lipoproteins cholesterol levels. Following the human study researchers conclude that withania is a potential source of hypoglycemic, diuretic and hypocholesterolemic agents. Six mild noninsulin-dependent diabetes mellitus subjects and six mild hypercholesterolemic subjects were treated with withania (6×500mg capsules per day (2 after every meal) accounting for a dose of 3g/day) for 30 days.⁸³

An animal study suggests that the aqueous extract of withania normalises hyperglycaemia in non-insulin-dependent diabetes mellitus (NIDDM) rats by improving insulin sensitivity. Treatment with withania reduced the elevated levels of blood glucose, glycosylated haemoglobin and insulin in the NIDDM rats. Withania treatment markedly improved the insulin sensitivity index that was significantly decreased in NIDDM control rats.⁸⁴

The activity of an ethanolic extract of withania is comparable to metformin, a known antiglycating

agent, a recent study has found. Therefore, scientists postulated that withania could have a therapeutic role in the prevention of glycation induced pathogenesis in diabetes mellitus and ageing. Modification of collagen such as non-enzymatic glycation and cross-linking plays an important role in diabetic complications and age-related diseases. The study evaluated the effect of withania on glucose-mediated collagen glycation and cross-linking *in vitro*.⁸⁵

Hypolipidaemic Activity

Hypocholesteraeamic and antioxidant effects of withania were investigated in hypercholesteraeamic rats. When the root powder of withania was added to the diet at 0.75 and 1.5gm/rat/day, hypercholesteraeamic animals registered significant decreases in total lipids, cholesterol and triglycerides in plasma. On the other hand, significant increases in plasma HDL-cholesterol levels, HMG-Coenzyme A reductase activity and bile acid content of liver were noted in these animals.

A similar trend was also noted in bile acid, cholesterol and neutral sterol excretion in the hypercholesteraeamic animals with withania administration. A significant decrease in lipid-peroxidation occurred in withania administered hypercholesteraeamic animals when compared to their normal counterparts. It appeared that withania is also effective in normal subjects for decreasing lipid profiles.⁸⁶

Drug Withdrawal

A recent study has demonstrated the therapeutic potential of withania as a valuable adjuvant agent in opioid dose-sparing therapies. Previous studies have demonstrated that withania prevents the development of tolerance to the analgesic effect of morphine (see below).⁸⁷ The study investigated whether withania extract (100mg/kg, intraperitoneal injection) may also modulate the analgesic effect induced by acute morphine administration (2.5, 5, 10mg/kg, beneath the skin) in experimental tests. Withania prolonged morphine-induced analgesia and suppressed the development of morphine-induced rebound hyperalgesia (increased sensitivity to pain) probably through involvement of GABAA, GABAB, NMDA and opioid receptors.⁸⁸

Results of a previous study indicates that pretreatment with withania protects from the

structural changes induced by morphine withdrawal potentially providing beneficial effects on the consequences related to this condition. Therefore treatment with withania in the weeks leading up to a planned opiate withdrawal may be beneficial.⁸⁹

Indications

- Anxiety, insomnia, stress especially with debility and nervous exhaustion, convalescence, fatigue, weakness, wasting disorders, especially in children and the elderly, emaciation, exhaustion associated with reduced iron levels, anaemia
- Longevity and conditions associated with aging such as memory loss, dementia, Alzheimer's disease, Parkinson's disease, cardiovascular disease (combines well with Terminalia arjuna)
- Chronic and degenerative diseases, especially of an inflammatory nature, such as arthritis, asthma, fibromyalgia, hypothyroidism, insulin resistance and psoriasis
- Reproductive health, low libido, impotence due to stress and poor vitality
- Possible prophylactic in cancer and adjunctive treatment during chemotherapy
- Assistance in the withdrawal of addictive drugs

Energetics

Warm, moist, sweet, astringent, bitter.¹¹

Use in Pregnancy

Withania is used as a tonic to support pregnancy and lactation, and to prevent miscarriage, in the Ayurvedic tradition. However Western texts urge caution in pregnancy due to reputed abortifacient activity and antifertility effects when taken in large doses which were noted in early animal studies despite there being no evidence of fetal damage.^{91,92}

Contraindications

Use with caution in peptic ulcer disease as withania may cause gastrointestinal irritation. People who are sensitive to the Solanaceae family should use this herb with caution.^{93,94,95}

Drug Interactions

There are reports that withania may potentiate the effects of barbiturates (central nervous system depressants) and benzodiazepines (a group of drugs called minor tranquillisers prescribed by a doctor to help people with anxiety or sleep problems e.g. diazepam – Valium; oxazepam – Serepax; nitrazepam – Mogadon; temazepam; flunitrazepam – Rohypnol) therefore caution should be used if taking this combination. It should be used under observation, and with caution by those taking thyroid hormones.^{96,97} Monitor with antihypertensive drugs. Combination may be beneficial with antipsychotic drugs such as haloperidol, clomipramine and chemotherapeutic agents. Medical supervision is recommended.

Administration and Dosage

Liquid Extract:	1:1
Alcohol:	45%
Weekly Dosage: ⁹⁸	15 to 40mL

References

- Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazón J. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules*. 2009 Jul 3;14(7):2373-93. doi: 10.3390/molecules14072373.
- The Plant List. [Internet]. Kew and Missouri: Royal Botanic Gardens, Kew and Missouri Botanical Garden; c2010 Version 1 [cited 2014 Feb 13] Available from <http://www.theplantlist.org/tpl1.1/record/kew-2465599>
- van Wyk B, Wink M. Medicinal Plants of the World. Pretoria: Briza Publications; 2004. p. 346.
- No authors listed. Monograph. *Withania somnifera*. *Altern Med Rev*. 2004 Jun;9(2):211-4.
- Purdie RW, Symon DE, Haegi L. Solanaceae. *Flora of Australia* 1982;29:184.
- Machiah DK, Girish KS, Gowda TV. A glycoprotein from a folk medicinal plant, *Withania somnifera*, inhibits hyaluronidase activity of snake venoms. *Comp Biochem Physiol C Toxicol Pharmacol*. 2006 Jun;143(2):158-61. Epub 2006 Mar 2.
- Ven Murthy MR, Ranjekar PK, Ramassamy C, Deshpande M. Scientific basis for the use of Indian ayurvedic medicinal plants in the treatment of neurodegenerative disorders: ashwagandha. *Cent Nerv Syst Agents Med Chem*. 2010 Sep 1;10(3):238-46.
- Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazón J. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules*. 2009 Jul 3;14(7):2373-93. doi: 10.3390/molecules14072373.
- Pratibha C, Madhumati B, Akarsh P. Therapeutic Properties and Significance of Different parts of Ashwagandha- A Medicinal Plant. *Int. J. Pure App. Biosci*. 2013;1 (6):94-101
- Singh N, Bhalla M, de Jager P, Gilca M. An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. *Afr J Tradit Complement Altern Med*. 2011;8(5 Suppl):208-13. doi: 10.4314/ajtcam.v8i5S.9. Epub 2011 Jul 3.
- Green, J. *The Male Herbal*. 2nd edn. Crossing Press: Berkeley 2007 p. 226.
- Misra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS, Tuli R. Withanolides from *Withania somnifera* roots. *Phytochemistry*. 2008 Feb;69(4):1000-4. Epub 2007 Dec 3.
- Pratibha C, Madhumati B, Akarsh P. Therapeutic Properties and Significance of Different parts of Ashwagandha- A Medicinal Plant. *Int. J. Pure App. Biosci*. 2013;1 (6):94-101
- No authors listed. Monograph. *Withania somnifera*. *Altern Med Rev*. 2004 Jun;9(2):211-4.
- Ganzera M, Choudhary MI, Khan IA. Quantitative HPLC analysis of withanolides in *Withania somnifera*. *Fitoterapia*. 2003 Feb;74(1-2):68-76.
- Singh N, Bhalla M, de Jager P, Gilca M. An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. *Afr J Tradit Complement Altern Med*. 2011;8(5 Suppl):208-13. doi: 10.4314/ajtcam.v8i5S.9. Epub 2011 Jul 3.
- Braun L, Cohen M. *Herbs and Natural Supplements*. 3rd ed. Sydney: Churchill Livingstone Elsevier; 2010. p. 1030.
- Pratte MA, Nanavati KB, Young V, Morley CP. An alternative treatment for anxiety: a systematic review of human trial results reported for the Ayurvedic herb ashwagandha (*Withania somnifera*). *J Altern Complement Med*. 2014 Dec;20(12):901-8. doi: 10.1089/acm.2014.0177.
- Choudhary D, Bhattacharyya S, Joshi K. Body Weight Management in Adults Under Chronic Stress Through Treatment With Ashwagandha Root Extract: A Double-Blind, Randomized, Placebo-Controlled Trial. *J Evid Based Complementary Altern Med*. 2017 Jan;22(1):96-106. Epub 2016 Apr 6.
- Biswal BM, Sulaiman SA, Ismail HC, Zakaria H, Musa KI. Effect of *Withania somnifera* (Ashwagandha) on the development of chemotherapy-induced fatigue and quality of life in breast cancer patients. *Integr Cancer Ther*. 2013 Jul;12(4):312-22. doi: 10.1177/1534735412464551. Epub 2012 Nov 9.
- Indian J Psychol Med. 2012 Jul;34(3):255-62. doi: 10.4103/0253-7176.106022. A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. Chandrasekhar K1, Kapoor J, Anishetty S.
- Andrade C, Aswath A, Chaturvedi SK, Srinivasa M, Raguram R. A double-blind, placebo-controlled evaluation of the anxiolytic efficacy of an ethanolic extract of *withania somnifera*. *Indian J Psychiatry*. 2000 Jul;42(3):295-301.
- Cooley K, Szczurko O, Perri D, Mills EJ, Bernhardt B, Zhou Q, et al. Naturopathic care for anxiety: a randomized controlled trial ISRCTN78958974. *PLoS One*. 2009 Aug 31;4(8):e6628. doi: 10.1371/journal.pone.0006628.
- Mishra SK, Trikamji B. A clinical trial with *Withania somnifera* (Solanaceae) extract in the management of sarcopenia (muscle aging). *Signpost Open Access J. Org. Biomol. Chem*. 2013; 1(1):187-194.
- Archana R, Namasivayam A. Antistressor effect of *Withania somnifera*. *J Ethnopharmacol*. 1999 Jan;64(1):91-3.
- Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol Biochem Behav*. 2003 Jun;75(3):547-55.
- Singh B, Chandan BK, Gupta DK. Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun. (Part II). *Phytother Res*. 2003 May;17(5):531-6.
- Mehta AK, Binkley P, Gandhi SS, Ticku MK. Pharmacological effects of *Withania somnifera* root extract on GABAA receptor complex. *Indian J Med Res*. 1991 Aug;94:312-5.
- Malhotra CL, Mehta VL, Prasad K, Das PK. Studies on *Withania ashwagandha*, Kaul. IV. The effect of total alkaloids on the smooth muscles. *Indian J Physiol Pharmacol*. 1965 Jan;9(1):9-15.
- Kumar A, Kalonia H. Effect of *Withania somnifera* on Sleep-Wake Cycle in Sleep-Disturbed Rats: Possible GABAergic Mechanism. *Indian J Pharm Sci*. 2008 Nov;70(6):806-10. doi: 10.4103/0250-474X.49130.
- Kumar A, Kalonia H. Protective effect of *Withania somnifera* Dunal on the behavioral and biochemical alterations in sleep-disturbed mice (Grid over water suspended method). *Indian J Exp Biol*. 2007 Jun;45(6):524 8.
- Pingali U, Pilli R, Fatima N. Effect of standardized aqueous extract of *Withania somnifera* on tests of cognitive and psychomotor performance in healthy human participants. *Pharmacognosy Res*. 2014 Jan;6(1):12-8. doi: 10.4103/0974-8490.122912.
- Chengappa KN, Bowie CR, Schlicht PJ, Fleet D, Brar JS, Jindal R. Randomized placebo-controlled adjunctive study of an extract of *withania somnifera* for cognitive dysfunction in bipolar disorder. *J Clin Psychiatry*. 2013 Nov;74(11):1076-83. doi: 10.4088/JCP.13m08413.
- Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, et al. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. *J Ethnopharmacol*. 2007 Jan 19;109(2):359-63. Epub 2006 Aug 4.
- Dhuley JN. Nootropic-like effect of ashwagandha (*Withania somnifera* L.) in mice. *Phytother Res*. 2001 Sep;15(6):524-8.

36. Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. *Neurochem Int*. 1997 Feb;30(2):181-90.
37. Bhatnagar M, Goel I, Roy T, Shukla SD, Khurana S. Complete Comparison Display (CCD) evaluation of ethanol extracts of *Centella asiatica* and *Withania somnifera* shows that they can non-synergistically ameliorate biochemical and behavioural damages in MPTP induced Parkinson's model of mice. *PLoS One*. 2017 May 16;12(5):e0177254. doi: 10.1371/journal.pone.0177254. eCollection 2017.
38. Patel A, Khan FA, Sikdar A, Mondal A, Shukla SD, Khurana S. Test for Non-Synergistic Interactions in Phytomedicine, Just as You Do for Isolated Compounds. *Journal of Experimental Neuroscience*. 2018;12:1179069518767654. doi:10.1177/1179069518767654.
39. Prakash J, Yadav SK, Chouhan S, Singh SP. Neuroprotective role of *Withania somnifera* root extract in maneb-paraquat induced mouse model of parkinsonism. *Neurochem Res*. 2013 May;38(5):972-80. doi: 10.1007/s11064-013-1005-4. Epub 2013 Feb 22.
40. Baitharu I, Jain V, Deep SN, Hota KB, Hota SK, Prasad D, et al. *Withania somnifera* root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *J Ethnopharmacol*. 2013 Jan 30;145(2):431-41. doi: 10.1016/j.jep.2012.10.063. Epub 2012 Dec 2.
41. Jain S, Shukla SD, Sharma K, Bhatnagar M. Neuroprotective effects of *Withania somnifera* Dunn. in hippocampal sub-regions of female albino rat. *Phytother Res*. 2001 Sep;15(6):544-8.
42. Tohda C, Kuboyama T, Komatsu K. Dendrite extension by methanol extract of *Ashwagandha* (roots of *Withania somnifera*) in SK-N-SH cells. *Neuroreport*. 2000 Jun 26;11(9):1981-5.
43. Parihar MS, Hemnani T. Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. *Biosci*. 2003 Feb;28(1):121-8.
44. Panda S, Kar A. *Withania somnifera* and *Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice. *J Ethnopharmacol*. 1999 Nov 1;67(2):233-9.
45. van der Hoof CS, Hoekstra A, Winter A, de Smet PA, Stricker BH. [Thyrotoxicosis following the use of ashwagandha]. [Article in Dutch] *Ned Tijdschr Geneesk*. 2005 Nov 19;149(47):2637-8.
46. Panda S, Kar A. Changes in thyroid hormone concentrations after administration of ashwagandha root extract to adult male mice. *J Pharm Pharmacol*. 1998 Sep;50(9):1065-8.
47. Gannon JM, Forrest PE, Roy Chengappa KN. Subtle changes in thyroid indices during a placebo-controlled study of an extract of *Withania somnifera* in persons with bipolar disorder. *J Ayurveda Integr Med*. 2014 Oct-Dec;5(4):241-5. doi: 10.4103/0975-9476.146566.
48. Gupta A, Singh S. Evaluation of anti-inflammatory effect of *Withania somnifera* root on collagen-induced arthritis in rats. *Pharm Biol*. 2014 Mar;52(3):308-20. doi: 10.3109/13880209.2013.835325. Epub 2013 Nov 5.
49. Sumantran VN, Chandwaskar R, Joshi AK, Boddul S, Patwardhan B, Chopra A. The relationship between chondroprotective and antiinflammatory effects of *Withania somnifera* root and glucosamine sulphate on human osteoarthritic cartilage *in vitro*. *Phytother Res*. 2008 Oct;22(10):1342-8. doi: 10.1002/ptr.2498.
50. Nagareddy PR, Lakshmana M. *Withania somnifera* improves bone calcification in calcium-deficient ovariectomized rats. *J Pharm Pharmacol*. 2006 Apr;58(4):513-9.
51. Henley AB, Yang L, Chuang KL, Sahuri-Arisoylu M, Wu LH, Bligh SW, et al. *Withania somnifera* Root Extract Enhances Chemotherapy through 'Priming'. *PLoS One*. 2017 Jan 27;12(1):e0170917. doi: 10.1371/journal.pone.0170917. eCollection 2017.
52. Senthilnathan P, Padmavathi R, Magesh V, Sakthisekaran D. Chemotherapeutic efficacy of paclitaxel in combination with *Withania somnifera* on benzo(a)pyrene-induced experimental lung cancer. *Cancer Sci*. 2006 Jul;97(7):658-64.
53. Agarwal R, Diwanay S, Patki P, Patwardhan B. Studies on immunomodulatory activity of *Withania somnifera* (*Ashwagandha*) extracts in experimental immune inflammation. *J Ethnopharmacol*. 1999 Oct;67(1):27-35.
54. Barua A, Bradaric MJ, Bitterman P, Abramowicz JS, Sharma S, Basu S, et al. Dietary supplementation of *Ashwagandha* (*Withania somnifera*, Dunal) enhances NK cell function in ovarian tumors in the laying hen model of spontaneous ovarian cancer. *Am J Reprod Immunol*. 2013 Dec;70(6):538-50. doi: 10.1111/aji.12172. Epub 2013 Nov 5.
55. Malik F, Kumar A, Bhushan S, Mondhe D, Pal H, Sharma R. Immune modulation and apoptosis induction: Two sides of antitumoural activity of a standardised herbal formulation of *Withania somnifera*. *European Journal of Cancer*; 2009May;45(8):1494-1509.
56. Sumantran VN, Boddul S, Koppikar SJ, Dalvi M, Wele A, Gaire V, et al. Differential growth inhibitory effects of *W. somnifera* root and *E. officinalis* fruits on CHO cells. *Phytother Res*. 2007 May;21(5):496-9.
57. Winters M. Ancient medicine, modern use: *Withania somnifera* and its potential role in integrative oncology. *Altern Med Rev*. 2006 Dec;11(4):269-77.
58. Mikolaj J, Erlandsen A, Murison A, Brown KA, Gregory WL, Raman-Caplan P, et al. *In vivo* effects of *Ashwagandha* (*Withania somnifera*) extract on the activation of lymphocytes. *J Altern Complement Med*. 2009 Apr;15(4):423-30. doi: 10.1089/acm.2008.0215.
59. Khan B, Ahmad SF, Bani S, Kaul A, Suri KA, Satti NK, et al. Augmentation and proliferation of T lymphocytes and Th-1 cytokines by *Withania somnifera* in stressed mice. *Int Immunopharmacol*. 2006 Sep;6(9):1394-403. Epub 2006 May 8.
60. Aphale AA, Chhibba AD, Kumbhakarna NR, Mateenuddin M, Dahat SH. Subacute toxicity study of the combination of ginseng (*Panax ginseng*) and ashwagandha (*Withania somnifera*) in rats: a safety assessment. *Indian J Physiol Pharmacol*. 1998 Apr;42(2):299-302.
61. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of *Ashwagandha*. *J Ethnopharmacol*. 1996 Feb;50(2):69-76.
62. Rasool M, Varalakshmi P. Antiperoxidative Effect of *Withania somnifera* Root Powder on Liver Lipid Peroxidation and Antioxidant Status in Adjuvant-induced Arthritic Rats. *Research Journal of Medicinal Plant*. 2008;2:28-33.
63. Nasimi Doost Azgomi R, Zomorodi A, Nazemyieh H, Fazljou SMB, Sadeghi Bazargani H, Nejatbakhsh F. Effects of *Withania somnifera* on Reproductive System: A Systematic Review of the Available Evidence. *Biomed Res Int*. 2018 Jan 24;2018:4076430. doi: 10.1155/2018/4076430. eCollection 2018.
64. Nasimi Doost Azgomi R, Nazemyieh H, Sadeghi Bazargani H, Fazljou SMB, Nejatbakhsh F, Moini Jazani A, et al. Comparative evaluation of the effects of *Withania somnifera* with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomised clinical trial. *Andrologia*. 2018 May 16:e13041. doi: 10.1111/and.13041. [Epub ahead of print]
65. Nasimi Doost Azgomi R, Zomorodi A, Nazemyieh H, Fazljou SMB, Sadeghi Bazargani H, Nejatbakhsh F. Effects of *Withania somnifera* on Reproductive System: A Systematic Review of the Available Evidence. *Biomed Res Int*. 2018 Jan 24;2018:4076430. doi: 10.1155/2018/4076430. eCollection 2018.
66. Shukla KK, Mahdi AA, Mishra V, Rajender S, Sankhwar SN, Patel D, et al. *Withania somnifera* improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations. *Reprod Biomed Online*. 2011 May;22(5):421-7. doi: 10.1016/j.rbmo.2011.01.010. Epub 2011 Mar 8.

67. Mahdi AA, Shukla KK, Ahmad MK, Rajender S, Shankhwar SN, Singh V, et al. Withania somnifera Improves Semen Quality in Stress-Related Male Fertility. *Evid Based Complement Alternat Med*. 2009 Sep 29. [Epub ahead of print]
68. Ahmad MK, Mahdi AA, Shukla KK, Islam N, Rajender S, Madhukar D. Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. *Fertil Steril*. 2010 Aug;94(3):989-96. doi: 10.1016/j.fertnstert.2009.04.046. Epub 2009 Jun 6.
69. Agarwal A, Nallella KP, Allamaneni SSR, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive BioMedicine Online*. 2004;8(6):616-627.
70. Desai N, Sharma R, Makker K, Sabanegh E, Agarwal A. Physiologic and pathologic levels of reactive oxygen species in neat semen of infertile men. *Fertility and Sterility*. 2009;92(5):1626-1631.
71. Kodama H, Kuribayashi Y, Gagnon C. Effect of sperm lipid peroxidation on fertilization. *Journal of Andrology*. 1996;17(2):151-157.
72. Sikka SC. Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*. 2001;8(7):851-862.
73. Maneesh M, Jayalekshmi H. Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. *Indian Journal of Clinical Biochemistry*. 2006;21(2):80-89.
74. Ambiyee VR, Langade D, Dongre S, Aptikar P, Kulkarni M, Dongre A. Clinical Evaluation of the Spermatogenic Activity of the Root Extract of Ashwagandha (*Withania somnifera*) in Oligospermic Males: A Pilot Study. *Evid Based Complement Alternat Med*. 2013;2013:571420. doi: 10.1155/2013/571420. Epub 2013 Nov 28.
75. Abdel-Magied EM, Abdel-Rahman HA, Harraz FM. The effect of aqueous extracts of *Cynomorium coccineum* and *Withania somnifera* on testicular development in immature Wistar rats. *J Ethnopharmacol*. 2001 Apr;75(1):1-4.
76. Ilayperuma I, Ratnasooriya WD, Weerasooriya TR. Effect of *Withania somnifera* root extract on the sexual behaviour of male rats. *Asian J Androl*. 2002 Dec;4(4):295-8.
77. Mishra RK, Verma HP, Singh N, Singh SK. Male infertility: lifestyle and oriental remedies. *Journal of Scientific Research*. 2012;56:93-101.
78. Owais M, Sharad KS, Shehbaz A, Saleemuddin M. Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine*. 2005 Mar;12(3):229-35.
79. Arora S, Dhillon S, Rani G, Nagpal A. The *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. *Fitoterapia*. 2004 Jun;75(3-4):385-8.
80. Shenoy S, Chaskar U, Sandhu JS, Paadhi MM. Effects of eight-week supplementation of Ashwagandha on cardiorespiratory endurance in elite Indian cyclists. *Journal of Ayurveda and Integrative Medicine*. 2012;3(4):209-214. doi:10.4103/0975-9476.104444.
81. Sood A, Mehrotra A, Dhawan DK, Sandhir R. Indian Ginseng (*Withania somnifera*) supplementation ameliorates oxidative stress and mitochondrial dysfunctions in experimental model of stroke. *Metab Brain Dis*. 2018 Apr 18. doi: 10.1007/s11011-018-0234-2. [Epub ahead of print]
82. Mohanty IR, Arya DS, Gupta SK. *Withania somnifera* provides cardioprotection and attenuates ischemia-reperfusion induced apoptosis. *Clin Nutr*. 2008 Aug;27(4):635-42. doi: 10.1016/j.clnu.2008.05.006. Epub 2008 Jul 11.
83. Andallu B, Radhika B. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root. *Indian J Exp Biol*. 2000 Jun;38(6):607-9.
84. Anwer T, Sharma M, Pillai KK, Iqbal M. Effect of *Withania somnifera* on insulin sensitivity in non-insulin-dependent diabetes mellitus rats. *Basic Clin Pharmacol Toxicol*. 2008 Jun;102(6):498-503. doi: 10.1111/j.1742-7843.2008.00223.x. Epub 2008 Mar 16.
85. Babu PV, Gokulakrishnan A, Dhandayuthabani R, Ameerthkhan D, Kumar CV, Ahamed MI. Protective effect of *Withania somnifera* (Solanaceae) on collagen glycation and cross-linking. *Comp Biochem Physiol B Biochem Mol Biol*. 2007 Jun;147(2):308-13. Epub 2007 Jan 31.
86. Visavadiya NP, Narasimhacharya AV. Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*. 2007 Feb;14(2-3):136-42. Epub 2006 May 18.
87. Kasture S, Vinci S, Ibba F, Puddu A, Marongiu M, Murali B, et al. *Withania somnifera* prevents morphine withdrawal-induced decrease in spine density in nucleus accumbens shell of rats: a confocal laser scanning microscopy study. *Neurotox Res*. 2009 Nov;16(4):343-55. doi: 10.1007/s12640-009-9069-2. Epub 2009 Jun 24.
88. Orrù A, Marchese G, Casu G, Casu MA, Kasture S, Cottiglia F, et al. *Withania somnifera* root extract prolongs analgesia and suppresses hyperalgesia in mice treated with morphine. *Phytomedicine*. 2014 Apr 15;21(5):745-52. doi: 10.1016/j.phymed.2013.10.021. Epub 2013 Nov 20.
89. Kasture S, Vinci S, Ibba F, Puddu A, Marongiu M, Murali B, et al. *Withania somnifera* prevents morphine withdrawal-induced decrease in spine density in nucleus accumbens shell of rats: a confocal laser scanning microscopy study. *Neurotox Res*. 2009 Nov;16(4):343-55. doi: 10.1007/s12640-009-9069-2. Epub 2009 Jun 24.
90. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev*. 2000 Aug;5(4):334-46.
91. Braun L, Cohen M. *Herbs and Natural Supplements*. 3rd ed. Sydney: Churchill Livingstone Elsevier; 2010. p. 1035.
92. No authors listed. Monograph. *Withania somnifera*. *Altern Med Rev*. 2004 Jun;9(2):211-4.
93. Braun L, Cohen M. *Herbs and Natural Supplements*. 3rd ed. Sydney: Churchill Livingstone Elsevier; 2010. p. 1035.
94. Gannon JM, Forrest PE, Roy Chengappa KN. Subtle changes in thyroid indices during a placebo-controlled study of an extract of *Withania somnifera* in persons with bipolar disorder. *J Ayurveda Integr Med*. 2014 Oct-Dec;5(4):241-5. doi: 10.4103/0975-9476.146566.
95. van der Hooft CS, Hoekstra A, Winter A, de Smet PA, Stricker BH. [Thyrotoxicosis following the use of ashwagandha]. [Article in Dutch] *Ned Tijdschr Geneesk*. 2005 Nov 19;149(47):2637-8.
96. Braun L, Cohen M. *Herbs and Natural Supplements*. 3rd ed. Sydney: Churchill Livingstone Elsevier; 2010. p. 1034.
97. No authors listed. Monograph. *Withania somnifera*. *Altern Med Rev*. 2004 Jun;9(2):211-4.
98. Williamson EM, editor. *Major Herbs of Ayurveda*. London (UK): Churchill Livingstone. 2002.