Radix Withaniae

Definition
Radix Withaniae consists of the dried roots of *Withania somnifera* (L.) Dunal. (Solanaceae) (*1*, *2*).

Synonyms
Physalis somnifera L. (*3*).

Selected vernacular names
Achuvagandi, agol, ahan, aksin, amukkuram, amukkaramkizangu, amukkira, angarberu, a sh a ga n dha, asagand, asagandh, asagandh nagori, asagandha, asan, asana, askagandha as'vagandha, ashvagandha, ashvakandika, ashwaganda, ashwagandha, ashwaganha, asgand, asgandh, asgandha, asganhisrol, asoda, asun, asundha, asunyho, asuvagandi, asvagandha, asvagandhi, aswagandha, aswal, aswgandh, babu, bâibru, bouzidân, dambarico, ghoda, ghodakun, ghodasan, gisawa, gizawa, hayagandhã, hidi-budawa, hirchil, e-gaddy, hiremaddina-gaddy, hiremaddina-gida, Indian ginseng, juustumari, kakani hindi, kaknaj-e-hindi, kilangee, kuvia, lakri, ol asajet, oroval, penneru, pennerugadda, punir, samoah, sebbere-gola, sim-alfirakh, sum-ul-far, sum-ul-firakh, techil, ubab, u'beb, ubuvimba, vajigandha, winter cherry, withania (*1–7*).

Geographical distribution
Widespread from the Mediterranean coast to India in semi-arid habitats (*4*, *8*).

Description
A woody herb or shrub, up to 2 m in height; growing from a long, tuberous taproot; stellatetomentose. Leaf: simple, 2–11 cm in length by 1.5–9.0 cm in width, exstipulate, petiole 6–20 mm long; blade elliptic to ovate-lanceolate, apex acute or rounded, base acute to long-decurrent, on vegetative shoots 8–10 cm long and alternate, on reproductive shoots 3–8 cm long and opposite, arranged in pairs of one large and one smaller

leaf; margin entire or wavy. Inflorescence: axillary, umbellate cyme of 2–25 yellow-green, short-pedicellate flowers. Flower: perfect, radially symmetrical, campanulate; calyx with 5 acute triangular lobes; corolla twice the length of the calyx, 7–8 mm long, with 5 lanceolate lobes, spreading or reflexed; stamens 5, slightly exserted, filaments alternate to petal lobes, partially fused to corolla; ovary superior, glabrous, stigma shallowly bifid. Fruit: berry; globose, 5–6 mm in diameter, orange-red, enclosed in green, membranous, inflated calyx approximately 2.5 cm in diameter and slightly 5-angled. Seeds: many, discoid, 2.5 mm in diameter, pale yellow (*4*, *9*).

Plant material of interest: dried root

General appearance

Straight and unbranched, the thickness varying with age. The main roots bear fibre-like secondary roots. The outer surface of the root is buff to grey-yellow with longitudinal wrinkles. The crown consists of 2–6 remains of the stem base. The base of the stem is green, variously thickened, cylindrical and longitudinally wrinkled. The roots break with a short uneven fracture (*1*, *2*).

Organoleptic properties

Odour: characteristic, horse-like; taste: sweetish, yet bitter and astringent and slightly mucilaginous (*1*, *2*).

Microscopic characteristics

The transverse section shows a narrow band of yellowish cork, exfoliated or crushed, a narrow cortex packed with starch grains; cork cambium of 2–4 diffused rows of cells; secondary cortex about 24 layers of compact parenchymatous cells; phloem consists of sieve tube, companion cells, phloem parenchyma; cambium 4–5 rows of tangentially elongated cells; secondary xylem hard, forming a closed vascular ring separated by multiseriate medullary rays; a few xylem parenchyma (*1*, *2*).

Powdered plant material

Dusty white or grey to yellow-brown. Cork thin-walled; lignified, cubical or elongated cells, often indistinct and collapsed, with yellowish-brown contents; 2–3 cells deep in smaller roots, up to 16 in larger primary roots. Parenchyma of the cortex composed of large thin-walled cells, packed with starch granules, and occasionally containing microsphenoidal crystals of calcium oxalate. Xylem elements are either tracheidal with bordered pits or, more rarely, reticulately thickened vessels. Fibres from xy-

lem have thickened lignified walls and simple pits. Starch abundant, simple or 2–4-compound, with a pronounced irregularly shaped hilum (*4*, *9*).

General identity tests

Macroscopic and microscopic examinations (*1*), thin-layer chromatography (*4*) and high-performance liquid chromatography (*10*).

Purity tests

Microbiological

Tests for specific microorganisms and microbial contamination limits are as described in the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*11*).

Foreign organic matter

Not more than 2.0% (*1*, *2*).

Total ash

Not more than 7% (*1*, *2*).

Acid-insoluble ash

Not more than 1% (*1*, *2*).

Water-soluble extractive

To be established in accordance with national requirements.

Alcohol-soluble extractive

Not less than 15% (*1*, *2*).

Loss on drying

To be established in accordance with national requirements.

Pesticide residues

The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*12*). For other pesticides, see the *European pharmacopoeia* (*12*) and the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*11*) and pesticide residues (*13*).

Heavy metals

For maximum limits and analysis of heavy metals, consult the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*11*).

Radioactive residues

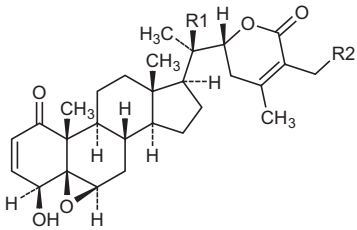
Where applicable, consult the WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (*11*).

Chemical assays

Contains not less than 0.2% of total alkaloids determined by gravimetric method (*1*).

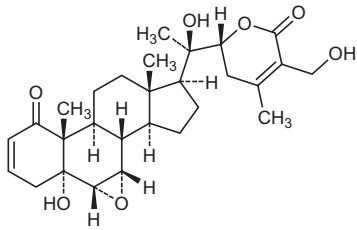
Major chemical constituents

The major characteristic constituents are steroidal lactones collectively known as "withanolides" including withaferin A, 27-deoxywithaferin A, withanolide D, withanosides I–XI, and withasomniferols A–C. Alkaloids constitute the other major group of compounds found in this plant material. Among the alkaloids found in the root are anaferine, anahygrine, cuscohygrine, dl-isopelletierine, 3-tropyltigloate, tropane-3-β-ol, 3-α-tigloyl-oxy-tropane and tropine. Also present are saponins including sitoindosides VII–X (*5*, *8*, *14*). The structures of withaferin A, withanolide D, withasomniferol A, cuscohygrine and dl-isopelletierine are presented below.

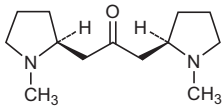


Withaferin A R1 = H R2 = OH

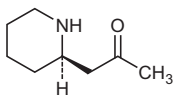
Withanolide D R1 = OH R2 = H



Withasomniferol A



Cuscohygrine



and enantiomer

Isopelletierine

Medicinal uses

Uses supported by clinical data

As an antistress agent to improve reaction time (*15*).

Uses described in pharmacopoeias and well established documents

As a general tonic to increase energy, improve overall health and prevent disease in athletes and the elderly (*1*, *16*).

Uses described in traditional medicine

Treatment of bronchitis, dyspepsia, impotency, scabies and ulcers (*5*, *16*).

Pharmacology

Experimental pharmacology

Antistress activity

The activity of a standardized extract of the root (1:1 aqueous ethanol fraction containing the withanolide glycosides and withaferin A at a concentration of 28–30%) was investigated in a rat model of chronic stress. A mild, unpredictable foot-shock was administered once daily for 21 days to rats. These chronic stress-induced perturbations were attenuated by the intragastric administration of the crude drug, at a dose of 25.0 and 50.0 mg/kg body weight (bw) given 1 h before foot-shock for 21 days (*17*).

In a mouse model of chronic stress, the antioxidant effects of the root were assessed in the forced swimming test. Biochemical analysis revealed that chronic swimming significantly increased lipid peroxidation and decreased glutathione levels in the brains of mice. The animal models also showed decreased levels of antioxidant defence enzymes, superoxide dismutase and catalase. Intragastric treatment with an extract of the crude drug, at a dose of 100.0 mg/kg bw significantly reduced lipid peroxidation and restored the glutathione levels decreased by chronic swimming in mice. Further, the treatment increased levels of superoxide dismutase in the forebrain and increased levels of catalase (*18*).

A withanolide-free aqueous fraction from the root (13 kg plant material in 70% ethanol, aqueous fraction) was evaluated for putative antistress activity. Intragastric administration of the preparation to immunocompromised mice for 7 days increased antibody production with a median effective dose of 40 mg/kg bw. Thus, the fraction exhibited significant antistress activity in a dose-related manner. The same fraction was protective against chemically and physically induced stress in rats and mice (*19*).

Intragastric administration of sitoindosides IX and X, at a dose range of 50–200.0 mg/kg bw also produced antistress activity in albino mice and rats, and augmented learning acquisition and memory retention in both young and old rats (*16*).

Anti-inflammatory activity

Numerous investigations have assessed the anti-inflammatory effects of the crude drug in vitro and in vivo (*20–22*). In one study, a suspension of powdered root (1 g/kg suspended in 2% acacia gum, 50 mg/ml) administered intragastrically to rats for 3 days, 1 hour before the injection of Freund's complete adjuvant, reduced inflammation. Many serum proteins such as α 2-glycoprotein, a major acute inflammatory phase protein and pre-albumin were decreased, indicating a reduction in acute inflammation (*20*). In another study by the same research group, 1 g/kg of the root suspension reduced the α 2-macroglobulin in the serum of rats given subplantar injection of carrageenan suspension (*21*).

In one study, air pouch granuloma was induced by subcutaneous injections of 4 ml of carrageenan on the dorsum of rats which had been subcutaneously injected 1 day previously with 6 ml of air on the dorsum (*22*). The powdered crude drug was administered by gastric lavage at a dose of 1 g/kg bw for 3 days. Radioactive sodium sulfate was injected intraperitoneally on day 9 and the incorporation of radio-labelled sulfur in glycosaminoglycan, oxidative phosphorylation (ADP/O ratio), mg^{2+}-dependent-ATPase enzyme activity and succinate dehydrogenase activity were determined in the mitochondria of the granuloma tissue. Administration of the root decreased the glycosaminoglycan content of the granuloma tissue by 92%, compared with 43.6% following treatment with hydrocortisone (15.0 mg/kg bw) and had no effect following treatment with phenylbutazone (100.0 mg/kg bw) (*22*).

In a further study, the effect of oral administration of the crude drug (root powder, 1 g/kg bw, daily for 15 days) on paw swelling and bony degenerative changes in Freund's complete adjuvant-induced arthritis in rats was assessed. Intragastric administration of the powdered root to the rats caused a significant reduction in both paw swelling and degenerative changes as observed by radiological examination. The reductions were better than those produced by the reference drug, hydrocortisone (15.0 mg/kg bw) (*16*, *23*).

Daily intragastric administration of 1.0 g/kg bw of the powdered root suspended in 2% gum acacia, was given 1 hour before the induction of inflammation by injection of Freund's complete adjuvant in rats for 3 days. Phenylbutazone (100.0 mg/kg bw) was administered to another group of animals as a positive control. Assessment of acute phase reactants of the blood showed changes in the concentration of α 2-glycoprotein, major acute phase α 1-protein, and pre-albumin in rats with inflammation, while in animals treated with the powdered root, the α 2-

glycoprotein and acute phase protein were decreased to undetectable levels (*16*).

Intragastric administration of the root, at doses of 500, 1000, 1500 or 1200 mg/kg bw, given orally as a suspension 3–4 hours prior to induction of inflammation, also caused a dose-dependent suppression of α 2-macroglobulin (an indicator for anti-inflammatory drugs) in the serum of rats inflamed by subplantar injection of carrageenan. The maximum effect (about 75%) was seen at 1.0 g/kg bw. Actual measurements of inflammation were not made (*16*). Glycowithanolides and a mixture of sitoindosides IX and X isolated from the crude drug were evaluated for their immunomodulatory and central nervous system effects (antistress and improvements to memory and learning) in mice and rats. Both mixtures of compounds led to mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes (*16*).

Anti-ischaemic activity

An in vivo study assessed the effect of the crude drug as a prophylactic treatment against stroke using the middle cerebral artery occlusion model in rats. Two groups of rats were pretreated with a hydroalcoholic extract of the crude drug (1.0 g/kg, administered by gastric lavage) for 15 or 30 days. The rats were then subjected to focal ischaemia by occlusion of the middle cerebral artery using an intraluminal thread. After 2 hours of middle cerebral artery occlusion, reperfusion was allowed by retracting the thread and the animals were assessed for ischaemic changes 30 min after reperfusion. Twenty-four hours later, rats were subjected to motor performance tests and were subsequently killed to enable estimation of the marker of oxidative stress, malondialdehyde. Significant motor impairment, with elevated levels of malondialdehyde, was observed in middle cerebral artery-occluded control rats. Treatment with the crude drug for 30 days prevented motor impairment and significantly decreased the raised levels of malondialdehyde compared with rats treated with the vehicle. Treatment also attenuated the percentage hemispheric lesion area in diffusion-weighted imaging (17 ± 2%) compared with the vehicle-treated middle cerebral artery-occluded group (30 ± 4%) (*24*).

A study was conducted to evaluate the cardioprotective potential of a hydro-alcoholic extract of the roots, by assessing the effects of treatment on haemodynamic, histopathological and biochemical parameters in isoprenaline- (isoproterenol-) induced myocardial necrosis in rats and to compare the effects with those of vitamin E (*25*). Rats were divided into six main groups: sham, isoprenaline control, *Withania somnifera* and vitamin E controls and *Withania somnifera* and vitamin E treatment groups. The root extract was administered at doses of 25, 50 and 100 mg/kg, and

vitamin E at a dose of 100 mg/kg, orally for 4 weeks. On days 29 and 30, the rats in the isoprenaline control group and the *Withania somnifera* and vitamin E treatment groups were given isoprenaline (85 mg/kg), subcutaneously at an interval of 24 hours. On day 31, haemodynamic parameters were recorded before the animals were killed, and the hearts were subsequently removed and subjected to histopathological and biochemical studies. A significant decrease in glutathione ($p < 0.05$), activities of superoxide dismutase, catalase, creatinine phosphokinase and lactate dehydrogenase ($p < 0.01$) as well as an increase in the level of the lipid peroxidation marker malonyldialdehyde ($p < 0.01$) was observed in the hearts of rats in the isoproterenol control group as compared to rats in the sham control group. Treatment with the root extract exerted a strong protective effect against isoprenaline-induced myonecrosis in rats. The dose of 50 mg/kg bw of the root extract had the greatest cardioprotective effect of the treatments studied (*25*).

Antioxidant activity

Dried ethanol extracts of the roots were tested for their total antioxidant activity in the iron (Fe^{3+}) reducing assay, and found to be potent reductants of Fe^{3+} at pH 5.5 (*26*). In an in vivo study, the powdered roots were assessed for their ability to protect neurons against excitotoxic lesions induced by kainic acid in mice. Mice were anaesthetized with ketamine and xylazine and kainic acid was administered by intra-hippocampal injections. The results showed an impairment of the function of the hippocampus region of brain after injection of kainic acid, and lipid peroxidation and protein carbonyl content were significantly increased in comparison with control animals ($p < 0.05$). The extract given 3 weeks prior to injections of kainic acid resulted in a decrease in neurotoxicity and measures of lipid peroxidation and protein carbonyl declined. The results of this study suggest that the crude drug mitigates the effects of excitotoxicity and oxidative damage in hippocampus by its antioxidative properties (*26*).

The glycowithanolides isolated from the crude drug were investigated for their preventive effect on the animal model of tardive dyskinesia, induced by once daily administration of the neuroleptic, haloperidol, for 28 days. Involuntary orofacial movements chewing movements, tongue protrusion and buccal tremors were assessed as parameters of tardive dyskinesia. Intragastric administration of 100.0 and 200.0 mg of the root, concomitantly with haloperidol, for 28 days inhibited the induction of the neuroleptic tardive dyskinesia (*27*).

The effects of the roots against oxidative stress in haloperidol-induced orofacial dyskinesia (haloperidol-induced vacuous chewing movements and tongue protrusion) were assessed in rats. Animals were treated for 21 days with intraperitoneal haloperidol (1 mg/kg); on day 22, vacuous chewing movements and tongue protrusions were counted during a 5-minute observation period. Coadministration of the extract (100–300 mg/kg bw) dose-dependently reduced haloperidol-orofacial dyskinesia. Biochemical analysis revealed that chronic treatment with haloperidol significantly increased lipid peroxidation and decreased forebrain levels of glutathione and the antioxidant defence enzymes, superoxide dismutase and catalase. Coadministration of the crude drug extract significantly reduced the lipid peroxidation and significantly reversed the decrease in forebrain superoxide dismutase and catalase levels, but had no significant effect on the haloperidol-induced decrease in forebrain glutathione levels (*28*).

The sitoindosides VII–X and withaferin A (glycowithanolide), were tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase, catalase and glutathione peroxidase levels in the rat brain frontal cortex and striatum. Active glycowithanolides of *Withania somnifera* (10.0 or 20.0 mg/kg bw) were administered by intraperitoneal injection once daily for 21 days to groups of six rats. Dose-related increases in all enzymes were observed; the increases were comparable to those seen following administration of deprenyl (2 g/kg bw intraperitoneally), indicating that the crude drug has an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties (*29*).

In another study, an aqueous suspension of the crude drug was evaluated for its effect on stress-induced lipid peroxidation in mice and rabbits (*30*). Levels of lipid peroxidation in the blood were increased by intravenous administration of lipopolysaccharides from *Klebsiella pneumoniae* and peptidoglycans from *Staphylococcus aureus*. Simultaneous intragastric administration of the extract (100.0 mg/kg bw) prevented an increase in lipid peroxidation.

In another study, the powdered root was administered to mice at a dose of 0.7 and 1.4 g/kg bw, for 15 and 30 days, to determine its effects on lipid peroxidation, superoxide dismutase and catalase activities. Thirty days of treatment produced a significant decrease in lipid peroxidation, and an increase in both superoxide dismutase and catalase activities (*31*). The effect of an extract of the crude drug on the regulation of lead toxicity and lipid peroxidative activity was investigated in liver and kidney tissues of rodents. Lead treatment of the animals for 20 days enhanced

hepatic and renal lipid peroxidation, whereas administration of the extract at doses of 0.7 g/kg bw and 1.4 g/kg bw together with equivalent doses of lead acetate for 20 days significantly decreased lipid peroxidation and increased the activities of antioxidant enzymes, such as superoxide dismutase and catalase (*32*).

An in vivo study examined the attenuating effect of extracts of the root and of aloe vera on prevention of hippocampal and cortical cell degeneration due to oxidative damage in mice with streptozotocin-induced diabetes. Doses of both plant extracts given to experimental animals were based on the evaluation of their total antioxidant activity and also their potency to reduce Fe^{3+}. Lipid peroxidation and protein carbonyl were assayed in both regions of the brain and the changes in memory and motor behavioural functions in diabetic and control mice were observed. The results showed a significant increase in lipid peroxidation and protein carbonyl in the hippocampus and cortical regions of mice with streptozotocin-induced diabetes, as well as a significant impairment in both motor and memory behavioural functions in diabetic mice. However, when diabetic mice were supplemented with the extracts of the root and with aloe vera, the oxidative damage in both brain regions was reduced as marked by a significant decline in both lipid peroxidation and protein carbonyl. The combination of extracts of root and aloe vera was more effective in reducing oxidative damage in brain regions than either of the plant extracts given alone. The combination of the extract and the aloe vera lowered the blood glucose level in mice with streptozotocin-induced diabetes. Memory impairment and motor dysfunction were also lessened by supplementation with the plant extracts (*33*).

Chemopreventive activity

The chemopreventive effect of an alcohol extract of the crude drug on 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin cancer was investigated in mice. The skin lesions were induced by the twice-weekly topical application of DMBA for 8 weeks to the shaved backs of mice. The alcohol extract was administered at the maximum oral tolerated dose of 400.0 mg/kg bw three times per week on alternate days 1 week before DMBA application and treatment was continued for 24 weeks. The results showed a significant decrease in incidence and average number of skin lesions in mice that received the extract compared to those treated with DMBA alone at the end of week 24. A significant impairment was noticed in the levels of reduced glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase in skin lesions of DMBA-treated control mice compared with vehicle-

treated mice. These parameters returned to near normal following administration of the crude drug to DMBA-treated mice (*34*).

Another chemopreventive study of an alcohol extract of the crude drug against 20-methylcholanthrene-induced fibrosarcoma tumours in mice was performed. A single subcutaneous injection of 20-methylcholanthrene into the thigh region of the mice produced a high incidence (96%) of tumours. Intragastric administration of the extract at a dose of 400.0 mg/kg bw (1 week before injecting 20-methylcholanthrene and continued until 15 weeks thereafter) significantly reduced the incidence and volume of tumours and enhanced the survival of the mice, compared with mice injected with 20-methylcholanthrene. The tumour incidence was also delayed in the group treated with the extract when compared with mice injected with 20-methylcholanthrene without pretreatment with the extract. Liver biochemical parameters revealed a significant modulation of reduced glutathione, lipid peroxides, glutathione S-transferase, catalase and superoxide dismutase in mice treated with the extract compared with mice injected with 20-methylcholanthrene (*35*).

Effects on memory and cognition

An aqueous suspension (100 mg/ml of powdered root suspended in 2% acacia gum and water) was assessed for its effects on improving short-term memory by reversing the effects of memory deficits induced by scopolamine and amnesia induced by electroconvulsive shock (*36*). Daily administration of 200 mg/kg bw of the suspension significantly reversed the scopolamine-induced delay in latency for the animals to reach the shock free zone and the number of errors in the passive avoidance step-down test ($p < 0.001$). Treatment of the animals with 100 mg/kg bw of the suspension produced a significant reduction in latency to reach the shock free zone ($p < 0.05$) (*36*).

Sitoindosides VII–X and withaferin A were isolated from an aqueous methanol extract of the roots. Rats were treated with 40 mg/kg bw (intraperitoneally) of an equimolar mixture of these compounds for 7 days and the anticholinesterase activity was determined in brain slices. Acetylcholinesterase activity was increased in the lateral septum and globus pallidus indicating a possible enhancement of cognition (*37*).

Withanolides isolated from the crude drug inhibited acetylcholinesterase and butyrylcholinesterase enzymes in a concentration-dependent fashion with IC_{50} values ranging between 20.5 and 85.2 μM for acetylcholinesterase and butyrylcholinesterase. Lineweaver-Burk as well as Dixon plots and their secondary replots indicated that the compounds were linear mixed-type inhibitors of acetylcholinesterase and non-competitive inhibitors of acetylcholinesterase with K_i (dissociation constant) values

ranging between 20.0 and 45.0 μM. All compounds were found to be non-competitive inhibitors of butyrylcholinesterase with K_i values ranging between 27.7 and 90.6 μM (*38*).

Immune stimulant activity

The effects of the root were investigated in mice with myelosuppression induced by cyclophosphamide, azathioprin or prednisolone (*39*). Administration of the root (100 mg/kg bw) prevented myelosuppression in mice treated with all three immunosuppressive drugs tested. A significant increase in haemoglobin concentration ($p < 0.01$), red blood cell count ($p < 0.01$), white blood cell count ($p < 0.05$), platelet count ($p < 0.01$), and body weight ($p < 0.05$) was observed in treated mice as compared with untreated (control) mice (*39*).

The effect of the crude drug on the cellular immune responses was studied in normal and in tumour-bearing animals. Intraperitoneal injection of five doses of an extract of the crude drug (20 mg/dose/animal) enhanced the proliferation of lymphocytes, bone marrow cells and thymocytes in response to mitogens. Both phytohaemagglutinin and concanavalin A mitogens administered concomitantly with crude drug treatment doubled the proliferation of lymphocytes, bone marrow cells and thymocytes. Splenocytes treated with the crude drug together with the mitogen led to a six-fold increase in lymphocyte proliferation. Natural killer cell activity was stimulated by the crude drug extract in both normal and tumour-bearing animals and it was found to be earlier than in the controls (48.92% cell lysis). Antibody-dependent cellular cytotoxicity was found to be enhanced in the group treated with the crude drug on the ninth day after treatment (65% cell lysis) (*40*). These authors have also reported that intraperitoneal administration of a 70% methanol extract of the roots to mice at a dose of 20 mg/animal enhanced total white blood cell count on day 10 after the administration of a single dose; bone marrow cellularity also increased, and the delayed-type hypersensitivity reaction (Mantoux test) was reduced (*41*).

The immunomodulatory effects of extracts of the crude drug (suspensions in carboxymethyl cellulose) were investigated in mice to measure their effects on immune hyper-reactivity. The animal models used included antibody-mediated immune hyper-reactivity, as seen in active paw anaphylaxis with disodium chromoglycate and cell-mediated immune hyper-reactivity using the delayed-type hypersensitivity model with cyclophosphamide. The immunomodulatory effect was assessed in IgE-mediated anaphylaxis as the reduction of ovalbumin-induced paw oedema in animals treated with the crude drug suspension at doses of 150 and

300.0 mg/kg bw. The positive control drug used was disodium chromoglycate. Potentiation of the delayed-type hypersensitivity reaction was observed in animals treated with cyclophosphamide at a dose of 20.0 mg/kg bw, and the crude drug suspensions at a dose of 300–1000.0 mg/kg bw. A significant increase in white blood cell counts and platelet counts was observed in animals treated with the crude drug. A protective effect against cyclophosphamide-induced myelosuppression was observed in animals treated with suspensions of the crude drug at doses of 300–1000.0 mg/kg bw, and a significant increase in white blood cell counts and platelet counts was observed. Cyclophosphamide-induced immunosuppression was counteracted by treatment with the same crude drug suspension. Treated animals showed an increase in haemagglutinating antibody responses and haemolytic antibody responses towards sheep red blood cells (*42*).

In a study in mice, intraperitoneal administration of a methanol extract of the root (20.0 mg/kg bw) was found to significantly reduce leukopaenia induced by treatment with cyclophosphamide. On the twelfth day the total white blood cell count in the group treated with cyclophosphamide was 3720 cells/mm^2 and that of the group that received cyclophosphamide together with the root was 6120 cells/mm^2. Treatment with the root and cyclophosphamide significantly increased the bone marrow cellularity (13.1×10^6 cells/femur) compared to the group treated with cyclophosphamide alone (8×10^6 cells/femur) ($p < 0.001$). Administration of the extract increased the number of alpha-esterase positive cells in the bone marrow of animals treated with cyclophosphamide, compared with the animals in the group treated with cyclophosphamide alone (687/4000 cells) (*43*).

The mechanism underlying the immunostimulant effect of a methanol extract of the root was investigated by assessing nitric oxide production in J774 macrophages (*44*). At concentrations of 1–256 µg/ml the extract produced a significant and concentration-dependent increase in nitric oxide production, an effect which was abolished by N(G)nitro-L-arginine methyl ester, a non-selective inhibitor of nitric oxide synthase; dexamethasone, an inhibitor of protein synthesis; and N(alpha-p)-tosyl-L-lysine chloromethyl ketone, an inhibitor of nuclear factor-kappa-β activation. In addition, Western blot analysis showed that the methanol extract increased, in a concentration-dependent fashion, expression of inducible nitric oxide synthase protein. The results suggest that the crude drug may induce the synthesis of inducible nitric oxide synthase expression, probably by acting at the transcriptional level, and the increased nitric oxide production by macrophages could account, at least in part, for the immunostimulant properties (*44*).

Neuroprotective activity

Eighteen compounds isolated from a methanol extract of the crude drug enhanced neurite outgrowth in human neuroblastoma cells. Double immunostaining was performed in rat cortical neurons using antibodies to phosphorylated nuclear factor-H as an axonal marker, and to mitogen activated protein kinase as a dendritic marker. In cells treated with withanolide A, the length of nuclear factor-H-positive processes was significantly increased compared with that of vehicle-treated cells, whereas the length of mitogen activated protein kinase-positive processes was increased by withanosides IV and VI. These results suggest that axons are predominantly extended by withanolide A, and dendrites by withanosides IV and VI (*45*).

The anti-parkinsonian effects of a root extract were investigated in rats pretreated with 100, 200 and 300 mg/kg bw of the root extract orally for 3 weeks. On day 21, 2 µl of 6-hydroxydopamine was infused into the right striatum while animals in the sham-operated group received 2 µl of the vehicle solvent. Three weeks after being injected with 6-hydroxydopamine, the rats were tested for neurobehavioural activity and 5 weeks after treatment they were killed for the estimation of lipid peroxidation, reduced glutathione content, activities of glutathione S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase, catecholamine content, dopaminergic D2-receptor binding and tyrosine hydroxylase expression. The root extract reversed all the parameters in a dose-dependent manner indicating a potential for preventing neuronal injury in Parkinson disease (*46*).

Toxicity

In one study of its effects on the central nervous system, a 2% aqueous suspension of the powdered root, an alkaloid-containing extract of the root prepared in 10% propylene glycol using 2% gum acacia as the suspending agent, was used to determine acute toxicity (*47*). The acute median lethal dose (LD_{50}) was 465.0 mg/kg bw in rats and 432.0 mg/kg bw in mice. In an antistress-effect study, the acute toxicity of aqueous-methanol extracts of the crude drug and equimolar combinations of sitoindosides VII and VIII (SG-I) and withaferin-A (SG-2) were studied. The acute LD_{50} of SG-1 and SG-2 administered intraperitoneally to mice was 1076.0 mg/kg bw and 1564.0 mg/kg bw, respectively (*48*).

In one study of the effects of chronic administration, the root was boiled in water and administered to rats in their drinking-water for 8 months while monitoring body weight, general toxicity, well-being, number of pregnancies, litter size and weight of progeny (*16*). The estimated dose given was 100.0 mg/kg bw per day. In the second part of

the study, an estimated dose of 200 mg/kg bw per day was given for 4 weeks as above while monitoring body temperature, body weight, cortisol value in heparinized plasma and ascorbic acid content of the adrenals. The liver, spleen, lungs, kidneys, thymus, adrenals and stomach were examined histopathologically and were all found to be normal. The initial average body weights of the animals in the group treated with the crude drug (100.0 mg/kg bw per day) and in the control group on day 1 were 91 g and 106 g, which, after 4 weeks, had increased to 185 g (103%) and 178 g (67.9%), respectively. The rats treated with the crude drug gained more weight than those in the control group (no p value given). The percentage weight gain after 8 weeks of the same *Withania somnifera* treatment was 227% for the animals in the treated group and 145.3% for those in the control group. The relative body weight gain was significantly greater in the group treated with the crude drug than in the control group ($p < 0.001$). While it is not clear when the rats were mated, the average weights of the progeny at 1 month of age were 70 g and 45 g in the crude-drug-treated and control groups, respectively, indicating healthier progeny in the treated group. In the 4-week study, the weight gain in the animals in the treated group was comparable to that of those in the control group. The body temperature of animals in the group treated with the crude drug was 1.7 °C lower than in the control animals. The treatment caused an increase in lung and liver weights and a decrease in adrenocortical activity as was evident from the reduction in adrenal weight and a significant reduction in plasma cortisol ($p < 0.001$). Histopathologically, all organs were normal, and no toxicity was observed (*16*).

Clinical pharmacology

A double-blind, placebo-controlled clinical trial assessed the effects of the root (250 mg twice daily) on psychomotor performance in 30 healthy volunteers (*15*). The effects were compared with those of *Panax ginseng* (100 mg twice daily). Test parameters included tapping, cancellation test, mental mathematical calculations, logical deductions, choice reaction times and auditory reactions. The performance of both groups was superior to that of subjects who received a placebo and the performance of subjects given the crude drug was superior to that of those given *Panax ginseng* after 40 days of treatment.

Adverse reactions

May cause nausea, vomiting and diarrhoea (*4*).

Contraindications
Due to the lack of safety data and the fact that the crude drug has been used in traditional medicine to induce abortion, its use during pregnancy or breastfeeding is contraindicated (*4*).

Warnings
No information was found.

Precautions
Drug interactions
The crude drug may potentiate the effects of barbiturates and reduce the effects of diazepam and clonazepam (*4*).

Carcinogenesis, mutagenesis, impairment of fertility
No information was found.

Pregnancy: non-teratogenic effects
See Contraindications.

Other precautions
No information was found.

Dosage forms
Crude drug, extracts and tinctures.

Posology
(Unless otherwise indicated)
Powdered crude drug: 3–6 g of the dried powdered root (*1*). Orally as an antistress agent: 250 mg twice daily (*15*).

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