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Withania somnifera (Ashwagandha): A Review

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ABSTRACT

Withania somnifera, a commonly used herb in Ayurvedic medicine. Although the review articles on this plant are already published, this review article is presented to compile all the updated information on its phytochemical and pharmacological activities, which were performed by widely different methods. Studies indicate ashwagandha possesses antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, antiinflammatory, antitumor properties. Various other effects like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection, sexual behaviour, tolerance and dependence have also been studied. These results are very encouraging and indicate this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects. Clinical trials using ashwagandha for a variety of conditions should also be conducted.

KEY WORDS: *Withania somnifera*, Withanolides, Phytochemistry, Pharmacological activities.

INTRODUCTION

Withania somnifera (WS), also known as ashwagandha, Indian ginseng, and winter cherry, it has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The roots of the plant are categorised as rasayanas, which are reputed to promote health and longevity by augmenting defence against disease, arresting the ageing process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing (1).

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

TAXONOMICAL CLASSIFICATION

Kingdom	*: Plantae, Plants;*
Subkingdom	*: Tracheobionta, Vascular plants;*
Super division	*: Spermatophyta, Seeds plants;*
Division	*: Angiosperma*
Class	*: Dicotyledons*
Order	*: Tubiflorae*
Family	*: Solanaceae*
Genus	*: Withania*
Species	*: somnifera Dunal*

Botanical description: WS is a small, woody shrub in the Solanaceae family that grows about two feet in height. It can be found growing in Africa, the Mediterranean, and India. An erect, evergreen, tomentose shrub, 30-150 cm high, found throughout the drier parts of India in waste places and on bunds. Roots are stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish or lubrid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The roots are the main portions of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring.

Parts used: Whole plant, roots, leaves, stem, green berries, fruits, seeds, bark are used.

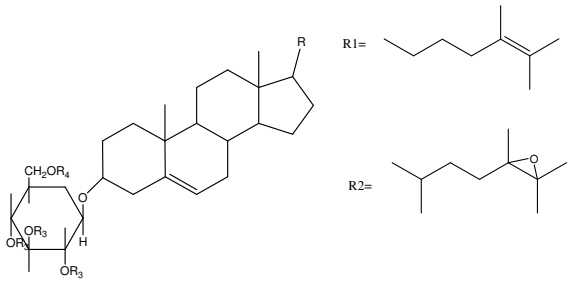
Synonyms:

Sanskrit: Ashwagandha, Turangi-gandha; ***English:*** Winter Cherry; ***Hindi:*** Punir, asgandh; ***Bengali:*** Ashvagandha; ***Gujrati:*** Ghodakun, Ghoda, Asoda, Asan; ***Telgu:*** Pulivendram, Panneru-gadda, panneru; ***Tamil:*** Amukkura, amkulang, amukkuram-kilangu, aswagandhi, ***Karnataka:*** Viremaddlinagadde, Pannaeru, aswagandhi, Kiremallinagida; ***Goa:*** Fatarfoda; ***Punjabi:*** Asgand, isgand; ***Bombay:*** Asgund, asvagandha; ***Rajasthani:*** Chirpotan

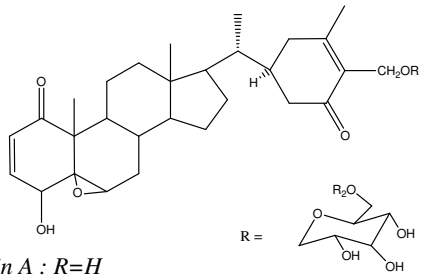
PHYTOCHEMISTRY

Chemical constituents of WS are always of an interest for the researchers. The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds, including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X) (3, 4). Apart from these contents plant also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of

amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron. Withaferin A, chemically characterized as 4b,27-dihydroxy- 5b-6b-epoxy-1-oxowitha-2, 24-dienolide, is one of the main withanolidal active principles isolated from the plant. WS showed chemogenetic variation and so far three chemotype I, II and III had been reported (5). These are chemically similar but differ in their chemical constituents especially in withanolide content. In Indian variety thirteen Dragendroff positive alkaloids have been obtained. The reported alkaloids are anaferine (bis (2-piperidylmethyl) ketone); isopelletierine; tropine; pseudotropine; 3α-tigloyloxtropine; 3- tropyltigloate; cuscohygrine; dl-isopelletierine; anahygrine; hygrine; mesoanaferine; choline; somniferine; withanine; withananine; hentriacontane; visamine; withasomnine, a pyrazole derivative from West Germany; pseudowithanine and ashwagandhine. Withaniol (mixture of withanolides) and number of withanolides including withaferine-A; withanolide N and O; withanolide D; withanolide p and 8; withanolide Q and R; withanolide y, 14α-hydroxy steroids and withanolides G, H, I, J, K and U (6). Seven new withanolide glycosides called withanosides I, II, III, IV, V, VI and VII had been isolated and identified (7). Much of WS pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. For physicochemical analysis, thin-layer chromatography (TLC) was used to identify the steroidal actones (withanolides) present in ashwagandha. The solvent system used was chloroform:methanol:water (64:50:10, v/v) and spots were finally identified with vanillin– phosphoric acid (8). The percentage of steroidal lactones was estimated spectrophotometrically.



Sitoindoside Vll : R=R_1 : R_3=H : R_4 = Palmitoyl
Sitoindoside Vlll : R=R_2 : R_3=H : R_4



Withaferin A : R=H
Sitoindoside lX: R_1 = D-glucoside; R_2=H
Sitoindoside X: R_1 = D-glucoside; R_2=Palmitoyl = Palmitoyl

Structures of key bioactive ingredients

PHARMACOLOGY

Although a lot of pharmacological investigations have been carried out based on the ingredients presents but a lot more can still be explored, exploited and utilized. A summary of the findings of these studies is presented below.

Antioxidant effect

The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. Free radical damage of nervous tissue may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum. Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue. Active glycowithanolides of WS were given once daily for 21 days, dose-related increased in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration. This implies that WS does have an antioxidant effect in the brain, which may be responsible for its diverse pharmacological properties (9). In another study, an aqueous suspension of WS root extract was evaluated for its effect on stress-induced lipid peroxidation (LPO) in mice and rabbits. LPO blood levels were increased by lipopolysaccharides (LPS) from *Klebsiella pneumoniae* and peptidoglycans (PGN) from *Staphylococcus aureus*. Simultaneous oral administration of WS extract prevented an increase in LPO (10). Apart from hepatic lipid peroxidation (LPO), the serum enzymes, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase, were assessed as indices of hepatotoxicity. Silymarin (20 mg/kg, p.o.) was used for comparison. Iron overload induced marked increase in hepatic LPO and serum levels of the enzymes, which was attenuated by glycowithanolides (WSG) in a dose-related manner, and by silymarin (11).

Anxiety and depression

Anxiolytic and antidepressant actions of the bioactive WSG, isolated from WS roots, in rats were assessed. WSG was administered orally once daily for 5 days and the results were compared by those elicited by the benzodiazepine lorazepam for anxiolytic activity, and by the tricyclic antidepressant, imipramine. WSG induced an anxiolytic effect was comparable to lorazepam, in the elevated plus-maze, social interaction and feeding latency in an unfamiliar environment, tests. WSG also reduced rat brain levels of tribulin, an endocoid marker of clinical anxiety, when the levels were increased following administration of the anxiogenic agent, pentylenetetrazole. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced 'behavioural despair' and 'learned

helplessness' tests. The investigations supported the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda (12).

Chronic stress

Chronic stress (CS) can result in a number of adverse physiologic conditions including cognitive deficit, immunosuppression, sexual dysfunction, gastric ulceration, irregularities in glucose homeostasis, and changes in plasma corticosterone levels. In a rat model of chronic stress WS and *Panax ginseng* extracts were compared for their ability to attenuate some effects of chronic stress. Both botanicals were able to decrease the number and severity of CS-induced ulcers, reverse CS-induced inhibition of male sexual behavior, and inhibit the adverse effects of CS on retention of learned tasks. Both botanicals also reversed CS-induced immunosuppression, but only the Withania extract increased peritoneal macrophage activity in the rats. The activity of the Withania extract was approximately equal to the activity of the *Panax ginseng* extract. WS, however, has an advantage over *Panax ginseng* in that it does not appear to result in ginseng- abuse syndrome, a condition characterized by high blood pressure, water retention, muscle tension, and insomnia (13). In another study, WS methanolic extract for 15 days significantly reduced the ulcer index, volume of gastric secretion, free acidity, and total acidity. A significant increase in the total carbohydrate and total carbohydrate/protein ratio was also observed. Study also indicated an increase in antioxidant defense, that is, enzymes SOD, CAT, and ascorbic acid, increased significantly, whereas a significant decrease in lipid peroxidation was observed. WS inhibited stress-induced gastric ulcer more effectively as compared to the standard drug ranitidine (14). In a study by Bhattacharya et al (15) chronic electroshock stress (14 days) significantly decreased the nor-adrenaline (NA) and dopamine (DA) levels in frontal Cortex, pons-medulla, hypothalamus, hippocampus and striatal, hypothalamal region, respectively, and increased the 5-hydroxytryptamine (5HT) level in frontal cortex, pons medulla, hypothalamus and hippocampus. Chronic stress also increased the rat brain tribulin activity. EuMil, a polyherbal formulation consisting WS as one of its ingredients for 14 days treatment normalized the perturbed regional NA, DA, 5HT concentrations, induced by chronic stress. EuMil also significantly attenuated the stress-induced increase in the rat brain tribulin activity.

Nootropic effect

Effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extract of roots of cultivated varieties of WS were studied on brain cholinergic, glutamatergic and GABAergic receptors in rats. The compounds slightly enhanced acetylcholinesterase (AChE) activity in the lateral septum and globus pallidus, and decreased AChE activity in the vertical diagonal band. These changes were accompanied by enhanced M1-muscarinic-cholinergic receptor binding in lateral and medial septum as well as in frontal cortices, whereas the M2- muscarinic receptor-binding sites were increased in a number of cortical regions including cingulate, frontal, parietal, and retrospinal cortex. The data suggest the compounds preferentially affect events in the cortical and basal forebrain cholinergic-signal transduction cascade. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition-enhancing and memory-improving effects of WS extracts in animals and in humans (16). In a study by Zhao et al (17) Withanoside IV (a constituent of WS; the root of WS) induced neurite outgrowth in cultured rat cortical neurons. Oral administration of withanoside IV significantly improved memory deficits in Abeta-injected mice and prevented loss of axons, dendrites, and synapses. Sominone, an aglycone of withanoside IV, was identified as the main metabolite after oral administration of withanoside IV. Sominone induced axonal and dendritic regeneration and synaptic reconstruction significantly in cultured rat cortical neurons damaged by Abeta. Withanoside IV may ameliorate neuronal dysfunction in Alzheimer's disease and that the active principle after metabolism is sominone. In another study reserpine treated animals also showed poor retention of memory in the elevated plus maze task paradigm. Chronic WS administration significantly reversed reserpine-induced retention deficits (18). In different study with WS root extract improved retention of a passive avoidance task in a step-down paradigm in mice. WS also reversed the scopolamine-induced disruption of acquisition and retention and attenuated the amnesia produced by acute treatment with electroconvulsive shock (ECS), immediately after training. Chronic treatment with ECS, for 6 successive days at 24 h intervals, disrupted memory consolidation on day 7. Daily administration of WS for 6 days significantly improved memory consolidation in mice receiving chronic ECS treatment. WS, administered on day 7, also attenuated the disruption of memory consolidation produced by chronic treatment with ECS. On the elevated plus-maze, WS reversed the scopolamine-induced delay in transfer latency on day 1. On the basis of these findings, it is suggested that WS exhibits a nootropic-like effect in naive and amnesic mice (19).

Antiparkinsonian properties

Parkinson's disease is a neurodegenerative disease characterized by the selective loss of dopamine (DA) neurons of the substantia nigra pars compacta. The events, which trigger and/or mediate the loss of nigral DA neurons, however, remain unclear. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for Parkinsonism. Administration of haloperidol or reserpine significantly induced catalepsy in mice. WS significantly inhibited haloperidol or reserpine-induced catalepsy and provide hope for treatment of Parkinson's disease (20). In another study, 6-Hydroxydopamine (6-OHDA) is one of the most widely used rat models for Parkinson's disease. There is ample evidence in the literature that 6-OHDA elicits its toxic manifestations through oxidant stress. Antiparkinsonian effects of WS extract has been reported due to potent antioxidant, antiperoxidative and free radical quenching properties in various diseased conditions. Rats were pretreated with the WS extract orally for 3 weeks. On day 21, 6-OHDA was infused into the right striatum while sham operated group received the vehicle. Three weeks after 6-OHDA injections, rats were tested for neurobehavioral activity

and were killed 5 weeks after lesioning for the estimation of lipidperoxidation, reduced glutathione content, activities of glutathione-S-transferase, glutathione reductase, GPX, SOD and CAT, catecholamine content, dopaminergic D2 receptor binding and tyrosine hydroxylase expression. WS extract reversed all the parameters significantly in a dose-dependent manner (21). In a study by Naidu et al (22) tardive dyskinesia is one of the major side effects of long-term neuroleptic treatment. The pathophysiology of this disabling and commonly irreversible movement disorder is still obscure. Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia. Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of tardive dyskinesia. Repeated treatment with reserpine on alternate days for a period of 5 days significantly induced vacuous chewing movements and tongue protrusions in rats. Chronic treatment with WS root extract for a period of 4 weeks to reserpine treated animals significantly and dose dependently reduced the reserpine-induced vacuous chewing movements and tongue protrusions. Oxidative stress might play an important role in the pathophysiology of reserpine-induced abnormal oral movements (22). In another study, WS glycowithanolides (WSG) administered concomitantly with haloperidol for 28 days, inhibited the induction of the neuroleptic TD. Haloperidol-induced TD was also attenuated by the antioxidant, vitamin E, but remained unaffected by the GABA-mimetic antiepileptic agent, sodium valproate, both agents being administered for 28 days like WSG. Antioxidant effect of WSG, rather than its GABA-mimetic action reported for the prevention of haloperidol-induced TD (23). WS significantly reversed the catalepsy, tardive dyskinesia and 6-Hydroxydopamine elicited toxic manifestations and may offer a new therapeutic approach to the treatment of Parkinson's disease.

Antivenom

Venom hyaluronidases help in rapid spreading of the toxins by destroying the integrity of the extra-cellular matrix of the tissues in the victims. A hyaluronidase inhibitor (WSG) is purified from WS. The glycoprotein inhibited the hyaluronidase activity of cobra (*Naja naja*) and viper (Daboia russelii) venoms, which was demonstrated by zymogram assay and staining of the skin tissues for differential activity. WSG completely inhibited the activity of the enzyme at a concentration of 1:1 w/w of venom to WSG. External application of the plant extract as an antidote in rural parts of India to snakebite victims appears to have a scientific basis (24). In a study by Lizano et al (25) antitoxin-PLA2 glycoprotein isolated from WS neutralized the PLA2 activity of the *Naja naja* venom. The implications of these new groups of PLA2 toxin inhibitors in snake biology as well as in the development of novel therapeutic reagents in the treatment of snake envenomations (25).

Antiinflammatory properties

The effects of WS, as antiinflammatory in a variety of rheumatologic conditions, have been studied by several authors. In a study, WS root extract (1 g/kg, oral) reduced Freund's complete adjuvant induced inflammation in rats; phenylbutazone was given as a positive control. The α2-glycoprotein found only in inflamed rat serum was decreased to undetectable levels in the WS group. Phenylbutazone, on the other hand, caused a considerable increase in the α2-glycoprotein in both arthritic and healthy rats (26). In another study, WS caused dose-dependent suppression of α2-macroglobulin (an indicator for antiinflammatory drugs) in the serum of rats inflamed by sub-plantar injection of carrageenan suspension (27). WS root powder also decreased air pouch granuloma induced by carrageenan on the dorsum of rats. WS decreased the glycosaminoglycans content in the granuloma tissue more than hydrocortisone treatment. WS also uncoupled the oxidative phosphorylation by significantly reducing the ADP/O ratio in mitochondria of granuloma tissue (28). In a different study, WS root extract (1000 mg/ kg, orally daily for 15 days) caused significant reduction in both paw swelling and bony degenerative changes in Freund's adjuvant-induced arthritis in rats as observed by radiological examination. The reductions were better than those produced by the reference drug, hydrocortisone (29). A study by al Hindawi et al (30) found WS inhibited the granuloma formation in cotton-pellet implantation in rats and the effect was comparable to hydrocortisone sodium succinate (5 mg/kg) treatment. In a double blind, placebo-controlled cross-over study, herbal formula significantly reduced the severity of pain and disability scores of patients with osteoarthritis (31). Few studies have been conducted on the mechanism of action for the antiinflammatory properties of WS. In one study, rats injected with formaline in the hind leg footpad showed a decrease in absorption of 14C-glucose in rat jejunum (32). Glucose absorption was maintained at the normal level by both WS and the cyclooxygenase inhibitor oxyphenbutazone. Both drugs produced antiinflammatory effects. Similar results were obtained in parallel experiments using 14C-leucine absorption from the jejunum (33). These studies suggest cyclooxygenase inhibition may be involved in the mechanism of action of WS.

Immunomodulation and hematopoiesis

The role of WS as immunomodulator has been extensively studied. In a mouse study, WS root extract enhanced total white blood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group (34). Recent research suggests a possible mechanism behind the increased cytotoxic effect of macrophages exposed to WS extracts. Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganisms and tumor cells. Iuvone et al demonstrated WS increased NO production in mouse macrophages in a concentration-dependent manner. This effect was attributed to increased production of inducible nitric oxide synthase, an enzyme generated in response to inflammatory mediators and known to inhibit the growth of many pathogens (35). In another study, Glycowithanolides and a mixture of sitoindosides IX and X isolated from WS, both produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis, and increased activity of the lysosomal enzymes. Root extract of WS was

tested for immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone (36).

Significant increases in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in WS-treated mice compared to untreated control mice. The authors also reported significant increases in hemolytic antibody responses toward human erythrocytes which indicated immunostimulatory activity. The effect of WS was also studied on the functions of macrophages obtained from mice treated with the carcinogen ochratoxin A (OTA). OTA treatment of mice for 17 weeks significantly decreased the chemotactic activity of the macrophages. Interleukin-1 (IL- 1) and tumor necrosis factor alpha (TNF-α) production was also markedly decreased (37).

In different study with the aqueous suspension of WS root powder was investigated for their *in vivo* and *in vitro* immunomodulatory properties. WS showed potent inhibitory activity towards the complement system, mitogen induced lymphocyte proliferation and delayed-type hypersensitivity reaction. Administration of WS root powder did not have a significant effect on humoral immune response in rats. Authors reported that immunosuppressive effect of WS root powder could be a candidate for developing as an immunosuppressive drug for the inflammatory diseases (38).

In a study of Gautam et al (39) Immunopotentiation on oral feeding of standardized aqueous extract of WS was evaluated in laboratory animals immunized with DPT (Diphtheria, Pertussis, Tetanus) vaccine. Treatment of immunized animals with test material for 15 days resulted in significant increase of antibody titers to *B. pertussis*. Immunized animals (treated and untreated) were challenged with B. pertussis 18,323 strain and the animals were observed for 14 days. Treated animals showed significant increase in antibody titers as compared to untreated animals after challenge. Immunoprotection against intracerebral challenge of live *B. pertussis* cells was evaluated based on degree of sickness, paralysis and subsequent death.

Reduced mortality accompanied with overall improved health status was observed in treated animals after intracerebral challenge of *B. pertussis* indicating development of protective immune response. In another study, WS also stimulated immunological activity in Balb/c mice. Treatment with five doses of WS was found to enhance the total WBC count on 10th day. Bone marrow cellularity as well as alpha-esterase positive cell number also increased significantly. Treatment with WS along with the antigen (SRBC) produced an enhancement in the circulating antibody titre and the number of plaque forming cells (PFC) in the spleen. Maximum number of PFC (985 PFC/10(6) spleen cells) was obtained on the fourth day. WS inhibited delayed type hypersensitivity reaction in mice (Mantoux test). Administration of WS also showed an enhancement in phagocytic activity of peritoneal macrophages when compared to control in mice. These results confirm the immunomodulatory activity of WS extract in indigenous medicine (40).

Antitumor properties

The chemopreventive effect was demonstrated in a study of WS root extract on induced skin cancer in mice given WS before and during exposure to the skin cancer causing agent 7,12-dimethylbenz[a]anthracene. A significant decrease in incidence and average number of skin lesions was demonstrated compared to the control group. Additionally, levels of reduced glutathione, SOD, CAT, and GPX in the exposed tissue returned to near normal values following administration of the extract. The chemopreventive activity is thought to be due in part to the antioxidant/ free radical scavenging activity of the extract (41). An *in vitro* study showed withanolides from WS inhibited growth in human breast, central nervous system, lung, and colon cancer cell lines comparable to doxorubicin. Withaferin A more effectively inhibited growth of breast and colon cancer cell lines than did doxorubicin. These results suggest WS extracts may prevent or inhibit tumor growth in cancer patients and suggest a potential for development of new chemotherapeutic agents (42). In another study WS was evaluated for its antitumor effect in urethane-induced lung adenomas in adult male albino mice. Simultaneous administration of WS (200 mg/kg daily orally for seven months) and urethane (125 mg/kg biweekly for seven months) reduced tumor incidence significantly. The histological appearance of the lungs of animals protected by WS was similar to those observed in the lungs of control animals. WS treatment also reversed the adverse effects of urethane on total leukocyte count, lymphocyte count, body weight, and mortality (43).

WS is widely used in the Ayurvedic system of medicine to treat tumors, inflammation, arthritis, asthma, and hypertension. Chemical investigation of the roots and leaves of this plant has yielded bioactive withanolides. Earlier studies showed that withanolides inhibit cyclooxygenase enzymes, lipid peroxidation, and proliferation of tumor cells. Several genes that regulate cellular proliferation, carcinogenesis, metastasis and inflammation are regulated by activation of nuclear factor-kappaB (NF-kappaB). Withanolides suppressed NF-kappaB activation induced by a variety of inflammatory and carcinogenic agents, including tumor necrosis factor (TNF), interleukin-1beta, doxorubicin, and cigarette smoke condensate. Suppression was not cell type specific, as both inducible and constitutive NF-kappaB activation was blocked by withanolides. The suppression occurred through the inhibition of inhibitory subunit of IkappaB alpha kinase activation, IkappaB alpha phosphorylation, IkappaB alpha degradation, p65 phosphorylation, and subsequent p65 nuclear translocation. NF-kappaB-dependent reporter gene expression activated by TNF, TNF receptor (TNFR) 1, TNFR-associated death domain, TNFR-associated factor 2, and IkappaB alpha kinase was also suppressed. Consequently, withanolide suppressed the expression of TNF-induced NF-kappaB-regulated antiapoptotic (inhibitor of apoptosis protein 1, Bfl-1/A1, and FADD-like interleukin-1beta-converting enzyme-inhibitory protein) and metastatic (cyclooxygenase-2 and intercellular adhesion molecule-1) gene products enhanced the apoptosis induced by TNF and chemotherapeutic agents, and suppressed cellular

TNF-induced invasion and receptor activator of NF-kappaB ligand-induced osteoclastogenesis. Overall, it is suggested that withanolides inhibit activation of NF-kappaB and NF-kappaB-regulated gene expression, which may explain the ability of withanolides to enhance apoptosis and inhibit invasion and osteoclastogenesis (44). In different study, the antiproliferative activity was screened against human laryngeal carcinoma (Hep2) cells by microculture tetrazolium assay (MTT). Two extracts (WS and WS-chloroform) and three fractions negatively affected Hep2 viability at the concentration and these were further investigated pharmacologically. Flow cytometry revealed cell cycle block and accumulation of hypoploid (sub G1) cells as the mode of antiproliferative activity. Their antiangiogenic potential was investigated by a chickchorio-allantoic membrane (CAM) wherein a significant inhibition of vascular endothelium growth factor (VEGF), induced neovascularization was recorded. The effect was confirmed *in vivo* by mouse sponge implantation method. These findings suggest that the roots of WS possess cell cycle disruption and antiangiogenic activity, which may be a critical mediator for its anticancer action (45). In a study of Senthilnathan et al (46) benzo(a)pyrene induced cancer animals were treated with WS extract for 30 days significantly alters the levels of immunocompetent cells, immune complexes and immunoglobulins. Based on the data, the carcinogen as well as the paclitaxel affects the immune system, the toxic side effects on the immune system is more reversible and more controllable by WS (47). In another study, a significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with WS. The hematological parameters were also corrected by WS in tumour-induced mice. These observations are suggestive of the protective effect of WS in Dalton's Ascitic Lymphoma (48). In different study with WS enhanced the proliferation of lymphocytes, bone marrow cells and thymocytes in responses to mitogens. Both PHA and Con A mitogens along with Withania treated splenocytes, bone marrow cells and thymocytes could stimulate proliferation twice greater than the normal. Withania treated splenocytes along with the mitogen LPS could stimulate the lymphocyte proliferation six times more than the normal. Natural killer cell activity (NK) was enhanced significantly in both the normal and the tumour-bearing group. Antibody dependent cellular cytotoxicity (ADCC) was enhanced in the Withania treated group on the 9th day. An early Antibody dependent complement mediated cytotoxicity (ACC) was observed in the WS treated group on day 13 (34). In a study of Gupta et al (48) after paclitaxel administration significant fall in total WBC and absolute neutrophil count was observed on day 3 and day 5. WS per se produced significant increase in neutrophil counts. WS when administered for 4 days before paclitaxel treatment and continued for 12 days caused significant reversal of neutropenia of paclitaxel. WS may be used as an adjuvant during cancer chemotherapy for the prevention of bone marrow depression associated with anticancer drugs.

Hypolipidemic effect

WS root powder decreased total lipids, cholesterol and triglycerides in hypercholesteremic animals. On the other hand, significantly increased plasma HDL-cholesterol levels, HMG-CoA reductase activity and bile acid content of liver. A similar trend also reported in bile acid, cholesterol and neutral sterol excretion in the hypercholesteremic animals with WS administration. Further, a significant decrease in lipid-peroxidation occurred in WS administered hypercholesteremic animals when compared to their normal counterparts. However, WS root powder was also effective in normal subjects for decreasing lipid profiles (49). In another study with aqueous extract of fruits of Withania coagulans to high fat diet induced hyperlipidemic rats for 7 weeks, significantly reduced elevated serum cholesterol, triglycerides and lipoprotein levels. This drug also showed hypolipidemic activity in triton-induced hypercholesterolemia. The histopathological examination of liver tissues of treated hyperlipidemic rats showed comparatively lesser degenerative changes compared with hyperlipidemic controls. The hypolipidemic effect of Withania coagulans fruits reported to be comparable to that of an Ayurvedic product containing Commiphora mukkul (50). In another study, hypoglycemic, diuretic and hypocholesterolemic effects of roots of WS were assessed on human subjects. Six mild NIDDM subjects and six mild hypercholesterolemic subjects were treated with the powder of roots of WS for 30 days. Suitable parameters were studied in the blood and urine samples of the subjects along with dietary pattern before and at the end of treatment period. Decrease in blood glucose was comparable to that of an oral hypoglycemic drug. Significant increase in urine sodium, urine volume, significant decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol were observed indicating that root of WS is a potential source of hypoglycemic, diuretic and hypocholesterolemic agents (51).

Sexual behaviour

Methanolic root extract of WS were orally administered at dose 3000 mg/kg/day of 7 days in rats. Their sexual behaviour was evaluated 7 days prior to treatment, day 3 and 7 of treatment, and day 7, 14 and 30 post-treatment by pairing each male with a receptive female. The WS root extract induced a marked impairment in libido, sexual performance, sexual vigour, and penile erectile dysfunction. These effects were partly reversible on cessation of treatment. This antimasculine effect was not due to changes in testosterone levels but attributed to hyperprolactinemic, GABAergic, serotonergic or sedative activities of the extract. WS roots may be detrimental to male sexual competence (52).

Antibacterial effect

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, as revealed by in vitro Agar Well Diffusion Method. The methanolic extract was further subfractionated using various solvents and the butanolic sub-fraction was possessed maximum inhibitory activity against a spectrum of bacteria including Salmonella

typhimurium. Moreover, in contrast to the synthetic antibiotic (viz. chloramphenicol), these extracts did not induce lysis on incubation with human erythrocytes, advocating their safety to the living cells. Oral administration of the aqueous extracts successfully obliterated salmonella infection in Balb/C mice as revealed by increased survival rate as well as less bacterial load in various vital organs of the treated animals (53). In another study, the methanol, hexane and diethyl ether extracts from both leaves and roots of WS were evaluated for the antibacterial/synergistic activity by agar plate disc-diffusion assay against *Salmonella typhimurium* and *Escherichia coli*. Different concentrations of Tibrim, a combination of rifampicin and isoniazid, were tested to find out the minimum inhibitory concentration (MIC), which came out to be 0.1 mg/ml for *S. typhimurium* and E. coli. From the six extracts tested, only methanol and hexane extracts of both leaves and roots showed potent antibacterial activity. A synergistic increase in the antibacterial effect of Tibrim was noticed when MIC of Tibrim was supplemented with these extracts (54).

Cardiovascular protection

WS may be useful as a general tonic, due in part to its beneficial effects on the cardiopulmonary system, as reported in the following studies. The effect of WS was studied on the cardiovascular and respiratory systems in dogs and frogs (55). The alkaloids had a prolonged hypotensive, bradycardiac, and respiratory-stimulant action in dogs. The study found that the hypotensive effect was mainly due to autonomic ganglion blocking action and that a depressant action on the higher cerebral centers also contributed to the hypotension. The alkaloids stimulated the vasomotor and respiratory centers in the brain stem of dogs. The cardio-inhibitory action in dogs appeared to be due to ganglion blocking and direct cardio-depressant actions. The alkaloids produced immediate predominant but short-lived cardio-depressant effects and a weak but prolonged cardiotonic effect in isolated normal and hypodynamic frog hearts. In another study, Left ventricular dysfunction was seen as a decrease in heart rate, left ventricular rate of peak positive and negative pressure change and elevated left ventricular end-diastolic pressure in the control group was recorded. WS showed strong cardioprotective effect in the experimental model of isoprenaline-induced myonecrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardioprotective effect (56).

Tolerance and dependence

Drug addiction, is one of the world's major health problem, with large direct health costs. Chronic treatment with benzodiazepine, ethanol or opioids induced tolerance and withdrawal signs. Benzodiazepine, ethanol and opioids induced tolerance and withdrawal also have blocked by a polyherbal preparation, BR-16A (Mentat), which has WS as a one of its ingredient (57-59). Interestingly, repeated administration of WS for 9 days attenuated the development of tolerance to the analgesic effect of morphine. WS also suppressed morphine-withdrawal jumps, a sign of the development of dependence to opiate as assessed by naloxone precipitation withdrawal on day 10 of testing (60). The studies revealed that the chronic administration of the WS did not exhibit any dependence-liability of its own, even upon an abrupt cessation. These findings may have clinical implications without producing tolerance and withdrawal effects on long-term use.

CONCLUSION

The extensive survey of literature revealed that WS is an important source of many pharmacologically and medicinally important chemicals, such as withaferins, sitoindosides and various useful alkaloids. In Indian variety thirteen Dragendroff positive alkaloids have been reported. The withanolides are the most searched chemical constituents of WS and till date around 138 withanolides with both ß and α side chain has been reported apart from various amino acid and other normal plant constituents. The plant has also been widely studied for their various pharmacological activities like antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, antiinflammatory, antitumor properties. Various other effects like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection, sexual behaviour, tolerance and dependence have also been studied. Although the results from this review are quite promising for the use of WS as a multi-purpose medicinal agent, several limitations currently exist in the current literature. While WS has been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use. It is also important to recognize that WS extracts may be effective not only on isolation, but may actually have a modulating effect when given in combination with other herbs or drugs.

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