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THERAPEUTIC POTENTIAL OF *WITHANIA SOMNIFERA*: A REPORT ON PHYTO-PHARMACOLOGICAL PROPERTIES

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ABSTRACT: *Withania somnifera* belongs to the Solanaceae family, commonly known as Ashwagandha, Indian ginseng or Winter cherry. It is an important commercial medicinal crop, considered as similar to *Panax ginseng* in Chinese medicine. Different parts of this plant are used in traditional medicine for the treatment of various ailments. It provides defense against diseases, adverse environmental factors and helps to retard the aging process. Ashwagandha exhibits a wide range of therapeutic properties by tuning the endocrine, cardiopulmonary, central nervous system and sexual behavior without any toxicity. The root has been used most frequently for therapeutic uses and is a constituent of over 200 formulations in Ayurveda, Siddha and Unani medicines. There are several reports to establish its immunomodulatory, anti-inflammatory, antistress, memory enhancing, antiparkinsonian, hypolipidemic, antibacterial, cardiovascular, antioxidant, antitumor and adaptogenic properties. These properties stem from the characteristic bioactive phytochemicals such as alkaloids and phytosterols present in the plant. This review presents a detailed survey on various traditional uses, phytochemical composition and pharmacological properties of *Withania somnifera*.

INTRODUCTION: Nature is a treasure chest of large diversity of medicinal plants. The search for drugs in nature by man dates from the far past, and this search resulted the evolution of different modern medicinal systems.

The curative powers of medicinal plants constitute the basis of all the indigenous systems of medicine namely Ayurveda, Unani, Siddha and Tibetan Medicine.

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Medicinal herbs have a great history in the realm of drug treatments, and are currently receiving attention as sources of synergistic combinations. Moreover medicinal plants made a lot of contribution towards the discovery of large number of new generation synthetic drugs. Recognition of the medicinal and the economic benefits of these plants are on increase in both developing and industrialized nations.

Thus, in all aspects of research and development, the exploration and evaluation of phyto-pharmacological effect of herbal drugs are relevant. Also we have to safely preserve the knowledge of medicinal plants and herbal remedies, which humankind has received from the past generations for posterity.

Withania somnifera (WS) belongs to the Solanaceae family, commonly known as Ashwagandha, Indian ginseng and Winter cherry. It got local names like Punir, asgandh (Hindi), Ashwagandha (Bengali), Ghodakun, Ghoda (Gujrati), Pulivendram (Telugu), Amukkura, amkulang (Tamil) etc. Ashwagandha in Sanskrit means "horse's smell" probably originated from the odor of its root, which resembles that of sweaty horse. The species name *somnifera* means "sleep-making" in Latin, attributed to sedating properties, but it has been used for sexual vitality and as adaptogenic properties also.

As a rasayana herb, the decoction and extracts of the herb shows excellent immunomodulatory activity by non-specific activation of macrophages, granulocytes, complement systems, natural killer cells and lymphocytes. It also influences the production of various effecter molecules generated by activated cells (para-immunity), gives protection against different pathogens including bacteria, fungi, viruses, etc., constitute an alternative to the conventional chemotherapy. The traditional uses of 'Ashwagandha' are to increase energy, youthful vigour, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat, blood, lymph, semen and cell production. It helps to counteract chronic fatigue, weakness, dehydration, bone weakness, impotency, premature aging emaciation, debility, convalescence and muscle tension. These pharmacological activities are mainly contributed by the steroidal alkaloids, withaferin A and withanolide D [1].

WS has been traditionally used for calming the mind and has the capacity to improve learning ability, memory power and to improve poor eye sight. WS shows anti-inflammatory potential in the treatment of joint diseases and an appropriate remedy for asthma and bronchitis. It is also used to inhibit the development of tolerance, dependence on chronic use of various psychotropic drugs and strengthening the female reproductive system.

Herbal combination containing Ashwagandha rejuvenates the body and is used in the treatment of infertility, impotence and seminal depletion. Combination with Shatavari has been used for the treatment of female infertility and frigidity and useful in threatened miscarriage, an excellent post-partum restorative as well as recommended to restore uterine tone [2, 3]. WS is reported to have antitumour, antioxidant, anti-inflammatory and immunoregulatory properties. The chemopreventive properties of WS make it a potentially useful adjunct for patients undergoing radiation and chemotherapy. It not only supports the health of patients undergoing conventional cancer treatment and but also cures the after effects such as carbuncles, ulcers and painful swellings [4]. The reports on the phyto-pharmacological studies justify its use in the traditional medicine. The review provides an outline of currently published research articles on the phytochemistry and pharmacology of *Withania somnifera*.

Plant Description: Ashwagandha grows as a stout, evergreen and tomentose shrub prolifically grows in drier parts of Asia, Africa, Congo, South Africa, Egypt, Morocco and Jordan [5]. It wildly grows in all drier parts of subtropical India and occurs in Madhya Pradesh, Uttar Pradesh, Punjab plains and northwestern parts of India like Gujarat and Rajasthan. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring. It is fairly easily grown plant, requires a warm sheltered position in full sun and a well-drained moderately fertile soil for cultivation.

It is an erect branching shrub that attains a height of 30-150 cm, covered in a wooly pubescence. The ovate leaves are up to 10 cm long and 2.5-5 cm wide, margins entire and arranged in an alternate fashion. The flowers are green or yellow, born in axillary fascicles, giving rise to red globose fruits when mature. Roots are 20-30 cm long and 6-12 mm in diameter, with few (2-3) lateral roots of slightly smaller size, straight and are unbranched. Outer surface is buff to grayish-yellow with longitudinal wrinkles and in the center soft, solid mass with scattered pores. It has a characteristic odor, taste bitter and is acrid. Whole plant, leaves, roots, stem, green berries, fruits, seeds and bark are used for therapeutic purpose, while roots are mostly utilized [2].

Phytochemical Profile: Phytochemicals are the non-nutritious chemical compounds present in plants, responsible for their medicinal properties. Extensive phytochemical studies of WS resulted in the isolation and identification of about 35

chemical constituents [6]. The main constituents of ashwagandha are alkaloids and steroidal lactones (Fig. 1-2). Alkaloids consist of withanine and other substituents such as somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, choline, cuscohygrine, isopelletierine and anaferine. The steroidal lactones includes ergostane type steroidallactones, withaferin A, withanolides A-Y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc.

The withanolides have C_{28} steroidal nucleus with C_9 side chain, with a six membered lactone ring. Presence of saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X) are also reported [7]. The plant shows variation in phytochemical composition with geographic distribution. Among the various alkaloids, withanine is the main constituent. In Indian variety thirteen Dragendroff positive alkaloids have been isolated. 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. These are chemically similar but differ in their chemical constituents especially in withanolide content [8].

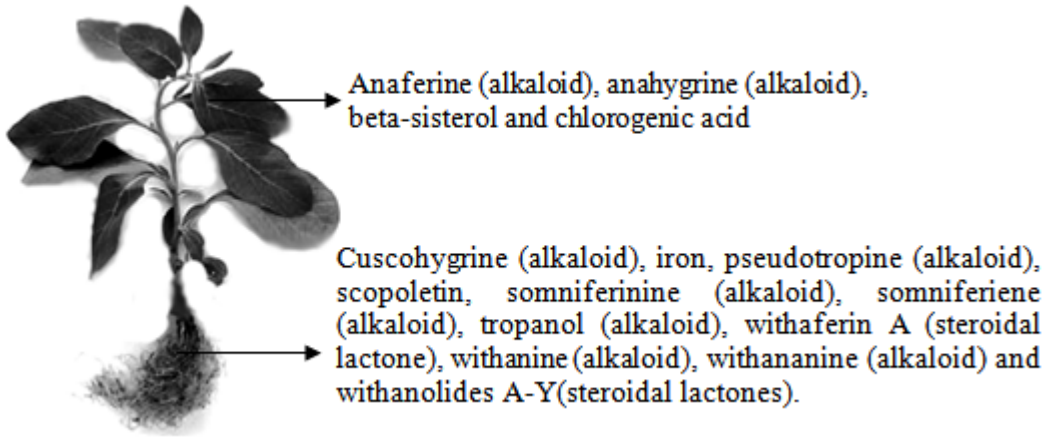
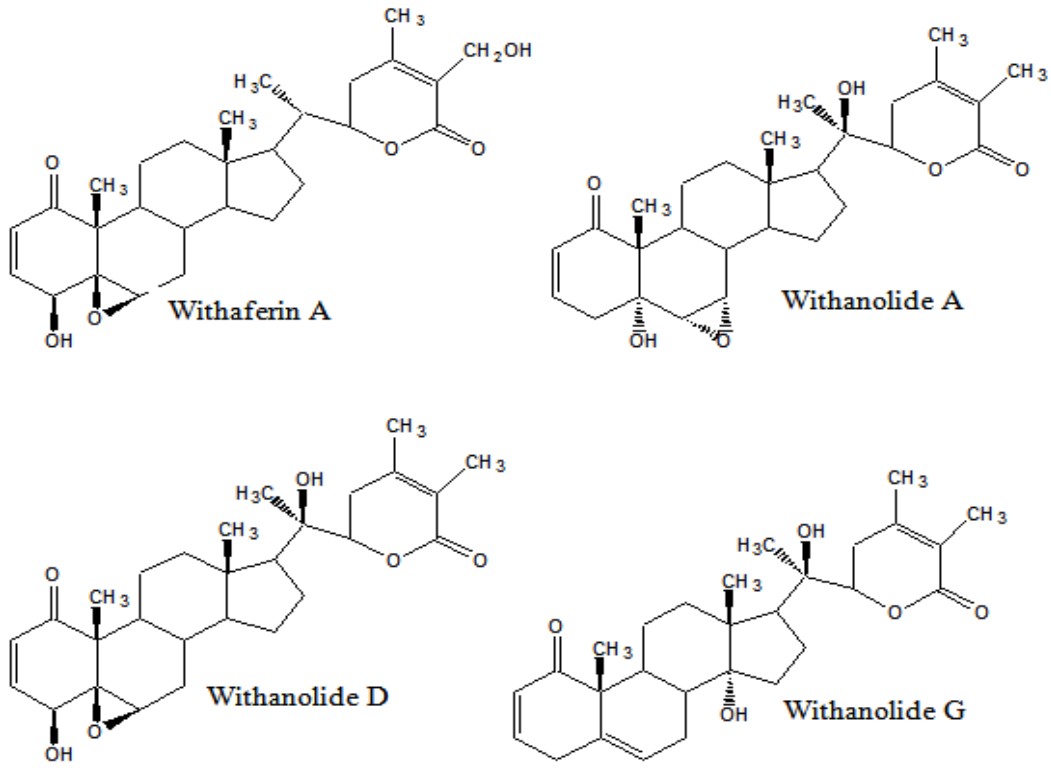


FIGURE 1: DISTRIBUTION OF MAJOR PHYTOCHEMICALS IN *WITHANIA SOMNIFERA*



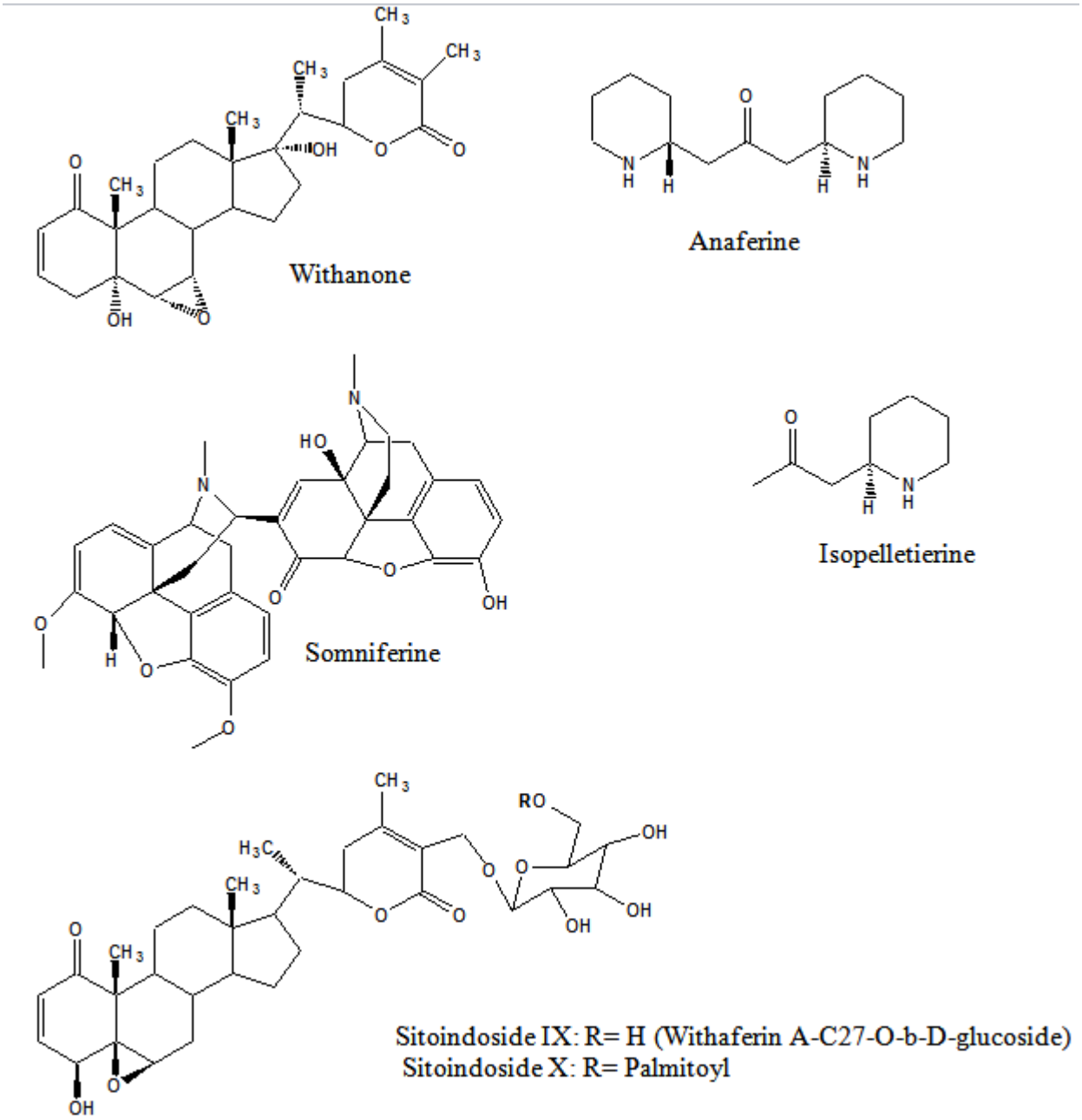


FIGURE 2: MAJOR BIOACTIVE PHYTOCHEMICALS PRESENT IN WS

Pharmacological Studies: Evaluation of *Withania somnifera* for its therapeutic potential by phyto-pharmacological studies proved that it possess adaptogenic, antibiotic, aboritifacient, aphrodisiac, astringent, anti-inflammatory, diuretic and sedative properties. It addition to this it can also provide potent antioxidant protection and stimulates the activation of immune system.

1. **Anti-Inflammatory Properties:** In Ayurveda *WS* is considered as an anti-inflammatory herb traditionally used for the treatment of arthritis and asthma. The inflammatory response is a complex cascade of steps that include an activation of white blood cells and the production and release of inflammatory mediators. WS reported to possess anti-inflammatory property in different pharmacological animal models of inflammation such as carrageenan-induced inflammation, cotton pellet granuloma and adjuvant-induced arthritis. It inhibited the granuloma formation in cotton-pellet implantation in rats similar to that of hydrocortisone sodium succinate [9-11]. Root extract of *WS* is found to be much effective against carrageenan induced air pouch granuloma on the dorsum of rats and it

decreased the glycosaminoglycans content in the granuloma tissue than hydrocortisone treatment [12]. WS extract also shows potent analgesic and antipyretic effect by retarding amplification and propagation of the inflammatory response in monosodium urate crystal-induced experimental rat models, without causing any gastric damage compared to indomethacin, a non-steroidal anti-inflammatory drug [13].

Herbal formulations of WS are effective in reducing the severity of pain and disability scores of patients with osteoarthritis without any toxic effect [14]. Withaferin A (WA), the biologically active steroid in the leaf extract, shows similar effect as hydrocortisone sodium succinate treatment in adjuvant-induced arthritis rat models, a close experimental approximation to human rheumatoid arthritis [15]. Administration of *W. somnifera* root powder to the collagen-induced 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 [16].

WA could significantly inhibit NFkappaB activation, which indicate that WA or WA-enriched *WS* extracts can be considered as a novel class of NFkappaB inhibitors, which hold promise as novel anti-inflammatory agents for treatment of various inflammatory disorders and cancer. It has been proposed that the antiproliferative, proapoptotic, anti-invasive, antiosteoclastogenic, antiangiogenic, anti-metastatic, radiosensitizing, antiarthritic, and cardioprotective effects assigned to withanolide may be mediated in part through the suppression of NF-kB and NF-kB-regulated gene products [17].

WS can inhibit cyclooxygenase (COX), the enzyme responsible for the formation of important biological mediators of inflammatory and anaphylactic reactions. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain [8]. The mechanism of action has been proved by the studies on the absorption of ^{14}C-glucose and ^{14}C-leucine in jejunum of rats injected with 3.5% formaline, which are treated with *WS* and the cyclooxygenase inhibitor, oxyphenbutazone [18, 19]. Another possible mechanism of action is its stabilizing action on lysosomal enzyme activity. *WS* root powder is found to be as effective as indomethacin in adjuvant arthritic model, there was significant suppression of paw swelling and lysosomal enzyme activity in the arthritic animals [20]. Thus the phyto-constituents of WS can be a promising anti-inflammatory and anti-arthritic drug by modulating the level of inflammatory mediators and enzymes without causing any side effects compared to other non-steroidal anti-inflammatory drugs.

2. **Antioxidant Property:** Antioxidants play an important protective role against free radicals or reactive oxygen species. Free radical damage of nerves may involved in normal aging process and neurodegenerative diseases like epilepsy, schizophrenia, Parkinson's and Alzheimer's diseases. *WS* extracts and its active principles withaferin A (glycowithanolides) and sito-indosides VII-X have the potential to modulate the activity of major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum. Glycowithanolides were found to normalize the augmented superoxide dismutase (SOD) and lipid peroxidation (LPO) activities. It has the capacity to enhance catalase (CAT) and glutathione peroxidase (GPX) activities in rat brain frontal cortex and striatum of chronic foot shock - stress induced models [21, 22].

Administration of *WS* root extract has significantly reduced the lipid peroxidation and restored the decreased glutathione, SOD and catalase levels in chronic reserpine treated rats [23]. Treatment with aqueous suspension of *WS* root extract could normalize the lipid peroxidation (LPO) levels increased by lipopolysaccharides from *Klebsiella pneumoniae* and peptidoglycans from *Staphylococcus aureus* in rabbits and mice models [18].

It is also an effective hepatoprotective drug similar to silymarin. It could effectively modulate the level of serum enzymes, alanine, aminotransferase, aspartate aminotransferase and lactate dehydrogenase [24].

WS extract inhibits both the lipid peroxidation and protein oxidative modification induced by copper in aging of Wistar rats. Premature aging associated with chronic nervous tension may be related to increased oxidative stress, which can be abolished by the potent antioxidant property of ashwagandha extract [25]. The antioxidant effect of active principles of *W. somnifera* may explain, at least in part, the reported anti-stress, cognition-facilitating, anti-inflammatory and anti-aging effects produced by them in experimental animals, and in clinical situations [26].

3. **Anticancer Properties:** Cancer is one of the major causes of death and there is an increase in cancer mortality in all ages. In the last century, great advances were made by modern medical system in cure and prevention of this disease, but none of the attempts were completely successful. Thus search for novel safe and effective therapies are still continuing and exploration of traditional medicine for their anticancerous effects are found to be promising.

   The phyto-constituents of *WS* are proved to have anti-carcinogenic, radiosensitizing and chemo-preventive properties in both *in vitro* and *in vivo* experimental models. *WS* also helps patients to recover from the adverse effects of chemotherapy. Skin cancer is one of the most common of all human cancers. The hydro-alcoholic root extract of WS showed a significant decrease in incidence and average number of skin lesions on 7, 12-dimethylbenz [α] anthracene (DMBA) induced skin cancer in Swiss albino mice.

   Further, there was significant modulation in the levels of glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase and glutathione [27]. Similar protective effect of WS extract was observed on two stage skin carcinogenesis induced by DMBA (dimethyl benzanthracene) and croton oil. It has also exhibited the potential to control the level of lipid peroxide. These studies indicate the chemopreventive potential and the ability to modulate the papilloma induced alterations to the antioxidant defense systems [28].

   1-oxo-5-beta; 6-beta-epoxy-witha-2-enolide, isolated from the root of *WS* has the potential to prevent the incidence of skin carcinoma induced by ultra violet radiation [29]. The chemo-preventive activity may be linked to the anti-oxidant/free radical-scavenging constituents of the WS extract along with its anti-inflammatory and immunomodulatory properties.

   WS extracts was found to significantly alter the level of leucocytes, lymphocytes, neutrophils, immune complexes and immunoglobulins (Ig) A, G and M in azoxymethane induced experimental colon cancer mice model and decreased the activities of TCA cycle key enzymes such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and alpha-keto glutarate dehydrogenase (alpha-KGDH) in colon cancer bearing animals [30, 31].

   Withanolides such as withaferin A shows antiproliferative activity on human tumor cell lines such as NCI-H460 (Lung), HCT-116 (Colon), SF-268 (CNS) and MCF-7 (Breast) [32]. Ashwagandhanolide, a dimeric withanolides with an unusual thio-ether linkage, isolated from the roots of *WS* displayed growth inhibition against human gastric (AGS), breast (MCF-7), central nervous system (SF-268), colon (HCT-116), and lung (NCI H460) cancer cell lines, with IC_{50} values in the range of 0.43-1.48 µg/ml [33].

   Ethanolic extracts of leaf, stem and root extracts of WS reported to have anti-proliferative activity against human cancer cell lines of different tissues, DU-145 (prostrate), HCT-15(colon), A-549 (lung) and IMR-32 (neuroblastoma) cell lines [34, 35]. It is found to be effective against human laryngeal carcinoma (Hep-2) cells when analyzed by microculture tetrazolium assay (MTT). It has cell cycle disruption and anti-angiogenic activity, which may be a critical mediator for its anti-cancer action. Flow cytometric studies shows that the mode of anti-proliferative activity can be cell cycle block and accumulation of hypoploid (sub G1) cells.

Their anti-angiogenic potential was also investigated by chick chorioallantoic membrane (CAM) test wherein a significant inhibition ($p<0.0001$) of vascular endothelium growth factor (VEGF), induced neovascularization was recorded. These effects were supportive to the observations of studies by sponge implantation method in mice models [36].

Withaferin A (WA), a promising anticancer constituent of *WS*, has been found to inhibit the growth of MCF-7 and MDA-MB-231, human breast cancer cells by causing apoptosis. The proapoptotic effect of this promising natural product is partially attenuated by p53 knockdown and E2-ER-α [37]. Animal studies confirmed that withanone can influence apoptosis signaling, p53 signaling, GM-CFS signaling, cell death receptor signaling and G2-M DNA damage regulation, which contributes to its anti-carcinogenic effects [38]. Withaferin A, which can act as a vimentin cytoskeleton inhibitor, has been found to be a potent breast cancer antimetastatic agent. The anti-metastatic activity of WA is, at least in part, mediated through its effects on vimentin and vimentin ser56 phosphorylation [39].

Phytoconstituents of *WS* can control the expression of nuclear NF-κB or suppressing intercellular tumor necrosis factor and potentiating apoptotic signaling in cancerous cell lines. It has been found that withanolides can suppress NF-κB activation induced by a variety of inflammatory and carcinogenic agents, including tumor necrosis factor (TNF), doxorubicin, and cigarette smoke condensate. This mechanism of action may explain the ability of withanolides to enhance apoptosis and inhibit invasion and osteoclastogenisis [40].

Withanolide sulfoxide, a novel bioactive compound obtained from methanolic extract of WS roots also found to suppress proliferation of human breast (MCF-7) cancer cell lines even at very low concentration. It can completely suppress TNF-induced NF-κB activation [41]. Suppression of NF-κB activation may play a key role in the antitumor action of *WS* since it is activated by carcinogens, tumor promoters, and inflammatory agents.

Deletion or mutation of the androgen receptor (AR) renders prostate tumors refractory to apoptosis by androgen ablation, the mainstay of prostate cancer therapy. Withaferin A induced Par-4-dependent apoptosis in androgen-refractory prostate cancer cells and regression of PC-3 xenografts in nude mice [42]. Withaferin A could exert potent anti-angiogenic activity *in vivo* at doses that are 500-fold lower than those previously reported to exert anti-tumor activity. Withaferin A also found to inhibit human umbilical vein endothelial cell (HUVEC) sprouting in three-dimensional collagen-I matrix at doses which are relevant to NF-κB inhibitory activity. The inhibition of NF-κB by withaferin A in HUVECs is supposed to be occurs by interference with the ubiquitin mediated proteasome pathway as suggested by the increased levels of polyubiquitinated proteins [43].

Withanolides can activate the apoptotic cascade through cytochrome C release from the mitochondria, inducing apoptotic cell death in leukemia cells [44]. Clinical trials using human cancer cell lines have shown that *WS* possesses anti-cancer and Th-1 immune up-regulatory activities. Study on the mechanism of action of *WS* as a cytotoxic agent has shown that it can induce apoptosis via the activation of intrinsic and extrinsic signaling pathways. It induced early generation of reactive nitrogen and oxygen species (RNOS) thus producing oxidative stress, mediated mitochondrial membrane potential (MMP) loss leading to release of cytochrome C to the mitochondria and apoptosis inducing factor to the nuclei of cells. Further it inhibited more than 50% tumor growth in mouse tumor models and selectively stimulated Th-1 immunity by enhancing the secretion of INF-γ and IL-2 [45].

Radiosensitizers makes tumor cells more sensitive to radiation therapy and are intended to enhance tumor cell killing with less effect on normal tissues. Alcoholic extract of *WS* root demonstrated increased effect of radiation on tumor regression as well as growth delay on Sarcoma-180 grown on the dorsum of adult BALB/c mouse.

Combination of abdominal gamma irradiation (RT, 75 Gy) with the drug schedules increased tumor cure and tumor-free survival [46]. Later it has been found that withaferin A (WA), isolated from the WS extract, inhibited tumor growth and increased tumor free survival in a dose dependent manner even in advanced tumors such as Ehrlich ascites carcinoma in adult Swiss albino mice [47, 48]. These results show that in addition to having a tumor inhibitory effect, the phytoconstituents of Ashwagandha can also acts as a radiosensitizer.

The Ayurvedic preparation Ashwagandha-rasayana is found to protect mice from cyclophosphamide (CTX) induced leucopenia and myelosuppression, one of the major drawbacks in cancer chemotherapy. Similarly treatment of *WS* extract along with CTX significantly ($P < 0.001$) increased the bone marrow cellularity and WBC count compared to CTX alone treated group. Thus *WS* has the potential to reduce the cyclophosphamide induced toxicity and cancer propagation [49, 50]. It is found to act as an adjuvant during cancer chemotherapy for the prevention of bone marrow depression associated with anticancer drugs based on its activity against paclitaxel induced neutropenia in mice.

Further, in a long term tumorigenesis study, *Withania* inhibited benzo (a) pyrene-induced forestomach papillomagenesis, in the Swiss albino mouse model. Similarly, Withania inhibited 7,12-dimethylbenzanthracene induced skin papillomagenesis without any apparent toxic effects [51, 52]. Treatment with *WS* root powder along with paclitaxel shows enhancement of antitumor effect of paclitaxel in benzo(a)pyrene induced experimental lung cancer models [53].

Reactivation of tumor suppressor genes that have been silenced by promoter methylation is a very attractive molecular target for therapy against cancer. In a recent report *Withania somnifera* extract found to show the reversal of hypermethylation of *RARβ2* gene. It was observed that the treatment of an adeno-carcinoma cervical cancer cell line, HeLa with 20 μg/ml of the ethanolic extract of *Withania somnifera* for 6 days resulted in demethylation of promoter of *RARβ2* gene, which elucidate an another mode of action [54].

Millions of people die by cancer despite of tremendous efforts to control and cure. The phyto-constituents of *WS* can reduce tumor cell proliferation thereby increasing overall animal survival time. Furthermore, it has been shown to enhance the effectiveness of radiation therapy and chemotherapeutic agents, potentially mitigates undesirable side effects. All these reports highlight the use of *Withania somnifera* as a novel complementary therapy for integrative oncology care and a source of effective lead molecule.

4. **Immunomodulatory Potential:** Immune system provides protection from invading pathogens and various cancers. The regulation of the immune system has been a major challenge for the management of autoimmune disorders, tumor immunity, infectious diseases and organ transplants. The immunomodulatory properties of *WS* are established fact and have been used in the formulations of 'rasayana', which makes the body resistant to diseases without any side effects.

WS extract could reduce the ovalbumin-induced paw edema in mice, almost similar to that of standard drug disodium chromoglycate [55]. It also shows protective effect in cyclophosphamide-induced myelosuppression by significant increase in white blood cell counts and platelet counts in mice. Treatment with extracts counteracted the Cyclophosphamide induced immunosuppression by significant increase in hemagglutinating antibody and hemolytic antibody responses towards sheep red blood cells [56].

BALB/C mice treated with *WS* extract showed significant inhibition of carcinogen ochratoxin A- induced suppression of chemotactic activity and productions of IL-1 and TNF-alpha by macrophages. In support to these findings, WS also reported to possess immunopotentiating and myeloprotective effect, which enhances the cytokine production, stem cell proliferation and its differentiation.

Administration of Withania extract resulted in significant increase (P<0.001) of bone marrow cellularity, total WBC count and α-esterase positive cell number [57].

Withania extract inhibits delayed type hypersentivity reaction (Mantoux test) and enhance the phagocytic activity of peritoneal macrophages in mice. *WS* modulate cell mediated immune responses in mice by enhancing the proliferation of lymphocytes, bone marrow cells and thymocytes in responses to mitogens [58]. Glycowithanolides and a mixture of sitoindosides IX and X isolated from *WS* demonstrated significant immunomodulatory potential in albino mice by showing positive effect on the mobilization and activation of peritoneal macrophages [59]. Possible mechanism of action of *WS* is likely to be that it may induce the synthesis of inducible nitric oxide synthase (NOS) expression by acting at transcriptional level based on the observation of effect of extract in J774 macrophages [60].

WS has the capacity to potentiate the cellular and humoral immune responses under immunesuppressed conditions, similar to that of levamisole. Oral administration of aqueous fraction of *WS* extract caused significant increase in the stress-induced depleted T-cell population and increased the expression of Th1 cytokines in chronically stressed mice [61]. Withaferin A (WA) and Withanolide E (WE) exhibits specific immunosuppressive effect on human B and T lymphocytes and on mice thymocytes. WE show specific effect on T lymphocytes whereas WA influences both B and T lymphocytes [62]. Oral feeding of standardized aqueous extract of *WS* demonstrated immunopotentiation in laboratory animals immunized with DPT vaccine and immunoprotection against intra-cerebral challenge of live *B. pertussis* cells [63]. It also shows immunoprotection in cancer chemotherapy [64].

Immu-21, a polyherbal formulation of *WS* is known for its immunomodulatory potential for modulating the proliferative response of splenic leukocytes to T cell and B cell mitogens. Pretreatment with Immu-21 selectively increased the proliferation of splenic leukocyte by B cell mitogen, LPS and cytotoxic activity against K 562 cells in mice. Immu-21 is also reported show antigenotoxicity as well as inhibition of both classical and non-classical chromosomal aberrations in mice induced by cyclophosphamide [65].

Modulation of cytokine secretion may offer novel approaches in the treatment of a variety of diseases. Immunomodulators alters the activity of immune function through the dynamic regulation of informational molecules such as cytokines. This may offer an explanation of the effects of herbs on the immune system and other tissues. *WS* and its combinations can modulate cytokines such as IL-1, IL-6, TNF, and IFN [66]. Thus these observations support the use of *WS* in traditional medicine as a rasayana herb.

5. **Adaptogeneic, Antidepressant and Anxiolytic Effect:** The traditional use of *WS* as an adaptogen has been assessed by different researchers and found that it can show better stress tolerance in cold water swimming tests, a classic experimental model of adaptogenic activity [67, 68]. The adaptogenic properties of Siotone, a herbal formulation of *WS*, has been proved by several experimental parameters like chronic stress, induced glucose intolerance, suppressed male sexual behavior, induced behavioral depression (Porsolt's swim despair test and learned helplessness test) and cognitive dysfunction (attenuated retention of learning in active and passive avoidance tests).

WS treatment can also influence the stress indices such as gastric ulceration, adrenal gland and spleen weights, ascorbic acid and corticosterone concentrations of adrenal cortex, and plasma corticosterone levels in albino rats [69, 70].

In another study by Bhattacharya and Muruganandam on chronic stress rat model, proved that WS has significant antistress adaptogenic activity, which confirms the clinical use of the plant in Ayurveda [71].

Ashwagandha demonstrates antistress adaptogenic potential, which is comparable with that of *Panax ginseng* extracts. However *WS* has some advantages over *Panax ginseng*, it does not show ginseng- abuse syndrome, a condition characterized by high blood pressure, water retention, muscle tension, and insomnia. Withanolide, isolated from free hydrosoluble fraction of *WS* roots exhibited significant anti-stress activity in a dose depended manner against a battery of tests such as hypoxia time, antifatigue effect, swimming performance time, swimming induced gastric ulceration and hypothermia [72].

EuMil, a polyherbal formulation consisting *WS* as one of its major ingredients has significant adaptogenic and anti-stress, activity, which was qualitatively comparable to *Panax ginseng*, against a variety of behavioral, biochemical and physiological perturbations, induced by unpredictable stress. EuMil also significantly attenuated the stress-induced increase in the rat brain tribulin activity [73].

Methanolic extract of *WS* root is found to be effective in stress-induced gastric ulcer when analysed by various models of gastric ulcer in rats such as ulcer induced by the indomethacin and swim (restraint) stress treatment. It significantly reduced the ulcer index, volume of gastric secretion, free acidity, and total acidity; the observations were comparable to those of the standard drug ranitidine [74]. Ashwagandha extract shows anxiolytic and antidepressive actions similar to that of commonly prescribed pharmaceuticals such as lorazepam. In another study, it showed antidepressant effect in forced swim-induced "behavioral despair" and "learned helplessness" tests, which was comparable with that of imipramine. *WS* treatment reduced the levels of tribulin, an endocoid marker of clinical anxiety in rat brain, which was augmented following the administration of the anxiogenic agent, pentylenetetrazole [19, 75].

The anti-anxiety effects of *WS* extracts may accounts to its GABA-like activity. Gamma Amino-butyric acid (GABA) is an inhibitory (calming) neurotransmitter in the brain. Its function is to decrease neuron activity and inhibit nerve cells from over firing. Too much neuronal activity can lead to restlessness and insomnia, but GABA inhibits the number of nerve cells that fire in the brain, and helps to induce sleep, uplift mood and reduce anxiety [76]. Human clinical trials to study the effects of a standardized extract of *WS* on elevated levels of the stress hormone cortisol were impressive.

Many of the adverse effects of stress are thought to be related to elevated levels of cortisol. The participants subjectively reported increased energy, reduced fatigue, better sleep, and an enhanced sense of well-being. The participants showed several measurable improvements, including a reduction of cortisol levels up to 26%, a decline in fasting blood sugar levels, and improved lipid profiles [77]. These reports support the traditional use of *WS* to stabilize mood in patients with behavioral disturbances and stress.

6. **Neuroprotective Effects:** Neurodegeneration is the progressive loss of structure or function of neurons, including death of neurons. Parkinson's, Alzheimer's and Huntington's diseases occur as a result of neuro-degenerative processes. Researchers found that ashwagandha can support the growth of nerve cell dendrites, which allow these cells to receive communications from other cells. Thus ashwagandha can heal the brain tissue changes that accompany dementia and also promote the growth of both normal and damaged nerve cells, suggesting that the herb may boost up healthy brain cell function as well as benefit diseased nerve cells [78-79]. *WS* extracts has also the potential to reduce the number of degenerating cells on stressed adult Swiss albino rats [80].

Alzheimer's disease is an irreversible, progressive brain disease that slowly destroys memory and thinking skills, and eventually even the ability to carry out the simplest tasks. The withanolide isolated from *WS*, inhibited acetylcholinesterase, an enzyme responsible for breaking down one of the brain's key chemical messengers in a concentration dependent fashion.

The cholinesterase inhibitory potential along with calcium antagonistic ability and safe profile in human neutrophil viability assay could make it a possible drug candidate for further study to treat Alzheimer's disease and associated problems [81, 82].

Parkinson's disease (PD) belongs to a group of conditions called motor system disorders, which are the result of the loss of dopamine-producing brain cells.*WS* extract showed anti-parkinsonian effects when evaluated by 6-Hydroxydopamine (6-OHDA) rat models. 6-OHDA elicits its toxic manifestations through oxidant stress. In the particular study, three weeks after 6-OHDA injections, rats were tested for neurobehavioral activity and evaluated the level of lipidperoxidation, 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.

WS extract was found to reverse all the parameters significantly in a dose dependent manner. Thus, the study demonstrates that the extract of *WS* may be helpful in protecting the neuronal injury in Parkinson's disease [83]. BR-16A (Mentat), a polyherbal formulation containing *WS* significantly reversed the haloperidol or reserpine-induced catalepsy in animal models, which offers a new therapeutic approach to the treatment of Parkinson's disease [84]. The potent antioxidant, antiperoxidative and free radical quenching properties of *WS* extract in various diseased conditions may contribute to its antiparkinsonian effects.

Tardive dyskinesia is a disorder that involves the involuntary rolling of the tongue and twitching of the face, trunk or limbs; often occurs in patients with Parkinsonism who are treated with phenothiazine. Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of tardive dyskinesia. Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia.

Treatment of *WS* root extract for a period of 4 weeks significantly reduced the vacuous chewing movements and tongue protrusions in a dose dependent manner [85, 86]. These findings provide tremendous hope that *WS* can be a treatment option for Parkinson's and Alzheimer's diseases, for which currently there is no cure.

Humans have an innate capacity to react to environmental stimulation which, by way of learning and experience, develops into a range of coordinated motor behaviors that are appropriate responses to the sensory information perceived by an individual. In the new study, a standardized, dried aqueous extract of ashwagandha roots and leaves (Sensoril®, Natreon Inc, USA), or a matching placebo was administered to healthy human subjects in a crossover study, with the goal of ascertaining its effects on cognition and psychomotor function. Subjects consumed 500 mg twice daily of either ashwagandha extract or placebo.

The researchers conducted a series of psychometric tests on subjects at baseline and at the end of the 2-week trial. Compared to those taking placebo, subjects taking ashwagandha showed a significant improvement at the end of the trial in the following tests: Reaction Time (measures attention and sensory motor performance), Digital System Substitution (measures attention, response speed, integration, and visual-motor coordination), Digit Vigilance Task (measures alertness, vigilance, and selective capacity), and Card Sorting (measures sensory, motor, integrative, and executive functions) [87].

A very recent study showed that four major withanamides present in the *W. somnifera* fruit extract crossed the blood–brain barrier in mice using HPLC coupled with high resolution quadrupole time of flight mass spectrometer (Q-TOF/MS) detection. Mice were administered with 250 mg/kg of *W. somnifera* extract by intraperitoneal injection, and the blood and brain samples analyzed by Q-TOF/MS detection [88].

These results may help to develop *W. somnifera* fruit extract as a preventive or therapeutic botanical drug for stress-induced neurological disorders having potential to tune sensory responses and coordination of psychomotor function.

7. **Cardioprotective and Hypocholesteremic:** Myocardial infarction and myocardial ischemia-reperfusion injury is a major public health burden of the current age and the leading cause of death in the world. *WS* is reputed as an ethnomedicine for cardiovascular diseases and found to increase the heart rate, contractility, relaxation and inhibition of lipid-peroxidation similar to that of vitamin E, a known cardioprotective antioxidant. Hydro-alcoholic extract of *WS* showed a strong cardioprotective effect on experimental model of isoprenaline-induced myocardial necrosis in Wistar albino 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 [89].

   WS can efficiently limit the myocardial injury after ischemia and reperfusion in Wistar rats. *WS* and Vit E favorably modulate most of the hemodynamic, biochemical and histo-pathological parameters. *WS* on chronic administration markedly augmented antioxidants (GSH, GSHPx, SOD, CAT), while Vit E could not stimulate the synthesis of endogenous antioxidants compared to sham [90]. CardiPro, a polyherbal formulation of *WS*, significantly protected the mice from doxorubicin induced cardiotoxic effects as evidenced by lower mortality (25%), less ascites, myocardial lipid peroxidation, normalization of antioxidant enzymes and minimal damage to the heart tissues [91].

   In addition to this, *WS* extracts also shows hypocholesteremic and antioxidant effects in hypercholesteremic male albino rats. It decreased the total plasma lipid level and a significant increase in HDL level, HMG-CoA reductase activity and bile acid content of liver in treated animals [92].

   Clinical studies conducted on human proved the potential of *WS* as hypoglycemic, diuretic and hypocholesterolemic drug.

   There was significant increase in urine sodium, urine volume, and decrease in serum cholesterol, triglycerides, LDL (low density lipoproteins) and VLDL (very low density lipoproteins) cholesterol without any adverse effects. There was also considerable decrease in blood glucose level in treated subjects, comparable to oral hypoglycemic drug [93]. Hyperglycemia is a major risk factor of cardiovascular diseases.

   The antioxidant property along with hypoglycemic and hypocholesterimic potential of WS makes it an effective cardioprotective drug.

8. **Antimicrobial Potential:** Medicinal plants represent a rich source of antimicrobial agents. *WS* extracts shows antibacterial activity against Gram-positive and Gram-negative bacteria [94]. In a particular study, crude extracts of *WS* showed antibacterial and antifungal potential against 20 bacterial and 17 fungal cultures. *Corynebacterium, Bacillus, Streptococcus* and *Staphylococcus* species were found to be highly susceptible to WS extracts. They also show protection against infection of *Aspergillus fumigates* in Aspergillosis Balb-C mice model. The probable mechanism underlying the protective action of Ashwagandha against systemic *Aspergillus* infection can be the potential to activate the macrophage function thereby phagocytosis [95].

   Methanol and hexane extracts of both leaves and roots of *WS* are found to be active against *S. typhimurium* and *E. coli* [96]. WS extracts are found to be safe compared to synthetic antibiotic (*viz*. chloramphenicol), as these extracts did not induce lysis of human erythrocytes, advocating their safety to the living cells [97]. Oral administration of the aqueous extracts 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.

Further a monomeric glycoprotein isolated from the *WS* root tubers was found to be effective against phytopathogenic fungi and bacteria [98]. Methanolic leaf extracts of *WS* is also reported to be effective against *B. subtilis, E. coli, P. fluorescens and S. aureus, A. flavus, D. turcica* and *F. verticillioides* [99]. There are several such recent reports on the antimicrobial potential of *WS* against human pathogens [100]. Thus the safety and efficacy of *WS* extracts makes it a suitable candidate for conventional and combinational therapy.

9. **Antimalarial Potential:** Malaria is a serious public health problem and resistance of *Plasmodium falciparum* to chloroquine has been reported in several countries. The cost and side effects of synthetic antimalarial drugs have led to renewed interest in the search of herbal medicines that have the potential to treat malaria with little or no side effects. *In vivo* investigation of antiplasmodial activity of *WS* extracts by the inoculation of rodent malaria parasite *Plasmodium berghei*, into Swiss albino mice intraperitoneally showed statistically significant percentage inhibition of parasitemia compared to the controls [101]. Interestingly, in combination with chloroquine, the hot water extracts of *W. somnifera* (root bark) extract showed statistically significant and improved suppressions in mice against chloroquine resistant *Plasmodium berghei* NK65 [102].

10. **Effect on Sexual Behavior:** In folk medicine Ashwagandha is considered as aphrodisiac and stress buster herbal drug. An aphrodisiac is a type of food or drink that has the effect of making sexually more arouse to those who consume it. *WS* is mentioned in the ancient *Kama Sutra* as an herb to be used for heightening sexual experience. *WS* has the ability to restore sexual health and improve overall vitality while promoting a calm state of mind. Studies show that *WS* can produce nitric oxide *in vivo*, which is known to dilate blood vessels. Aqueous extract of *WS* can elicits changes in pituitary gonadotropins coupled with an enhancement in epididymal sperm pattern in adult male rats and folliculogenesis in immature female rats [103].

WS treatment induced testicular development and spermatogenesis in immature Wistar rats by directly affecting the seminiferous tubules [104]. Treatment with *WS* extracts can improves prosexual behavior (chasing, nosing, and genital sniffing) of sexually sluggish mice as well as it increases testicular daily sperm production and serum testosterone level. Further, *WS* treatment reverted back the adverse effects of cadmium on seminiferous tubules, and motility and density of cauda epididymidal spermatozoa in infertile Parkes (P) strain mice [105].

WS extract can modulate sexual functions in streptozotocin (STZ) induced type 1 diabetic male Wistar rats. It is effective in lowering FSH serum level of treated animals compared to controls in both diabetic and non-diabetic groups, whereas progesterone, testosterone and LH levels were significantly higher in non-diabetic treated animals. Root extract was also able to reverse the reductive effect of diabetes on the progesterone. Thus *WS* may have a regulatory effect on diabetes-induced change of the levels of gonadal-hormones, especially progesterone, in male rats [106].

W. somnifera is also an effective drug for the treatment of male infertility. In a particular human trial, it has been found that the treatment with *WS* root powder resulted in stress busting and improvement of anti-oxidants level and overall semen quality in a significant number of individuals. Treatment resulted in 14% pregnancy in the partners of the patients. Improvement in the hormone imbalance can be the major contributing factor to fertility improvement [107].

11. **Other Therapeutic Properties:** In addition to the general medicinal properties, *WS* traditionally used in the treatment of several other human ailments like osteoporosis. Osteoporosis, characterized by reduction in bone density, is a significant reason of mortality among the elderly, particularly in oestrogen-deficient women. Ethanolic extract of *WS* root, which contains oestrogen-like withanolides, is found to be effective for antiosteoporotic activity on female Sprague-Dawley rat model

(either sham operated or ovariectomized). It was also found to be effective in prevention and ameliorate the symptoms of osteoporosis [108]. *W. sominifera* extract offers protection against urotoxicity induced by cyclophosphamide on animal models when assessed by morphological analysis of the bladders for inflammation, dark coloration, protein level in serum, glutathione (GSH) content in both bladder and liver and histopathological analysis of the bladder for necrotic damage. The drug treated animal showed normal conditions compared to the control [109].

Withaferin A shows significant protective effect against CCl_4-induced hepatotoxicity in rats, which was as effective as hydrocortisone [6, 15]. It can also act as nephroprotective drug against gentamicin induced nephrotoxicity in Wistar albino rats. The particular study concluded that ashwagandha root extract may have some role in decreasing serum urea and creatinine levels and normalize the kidney weight against gentamicin toxicity, which may be due to its ability to inhibit free radicals [110].

In rural parts of India, *WS* extract is used for external applications as an antidote to snakebite. The glycoprotein present in *WS* can inhibit 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 [111]. The aqueous extract of WS has the ability to neutralize the phospholipases A2 (PLA2) activity of the Naja naja venom [112]. A phospholipase inhibitor purified from *WS* by gel-filtration and ion-exchange chromatography was effectively neutralized the enzyme activity and pharmacological properties such as cytotoxicity, edema, and myotoxicity of a multi-toxic Indian cobra venom phospholipase [113].

Patents related to Ashwagandha: *WS* has been traditionally used in Ayurvedic system as an adaptogen, aphrodisiacs, diuretics and a memory enhancer. Seven American and four Japanese firms have filed for patents on formulations containing extracts of the herb Ashwagandha.

The Japanese patent applications are related to the use of the herb as a skin ointment and for promoting reproductive fertility. The U.S based company Natreon has also obtained a patent for an Ashwagandha extract. Another US establishment, the New England Deaconess Hospital, has taken a patent on an Ashwagandha formulation, which is claimed to alleviate symptoms associated with arthritis [114]. The list of patents accepted or granted is given in **Table 1**.

CONCLUSION: Ashwagandha is one among the wide diversity of the medicinal plant, which is exploited well for its phyto-pharmacological effect. The medicinal properties of *Withania somnifera* is available both in the written and non-written format as traditional knowledge since time immemorial. In traditional system, the plant has been used as an anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory and adaptogenic drug. It also exerts a positive influence on the endocrine, cardio-pulmonary, and central nervous systems with little or no associated toxicity. It has the capacity to fight cancers by reducing tumor size and proved to be a good natural source of a potent and relatively safe radiosensitizer/chemo-therapeutic agent.

Clinical trials and animal research support the use of ashwagandha for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease. The pharmacological activity of *WS* is attributed to the presence of several bioactive secondary metabolites namely Withaferin A, Withanolide, etc. Traditional knowledge regarding the usage of this plant is many but the scientific research available today to support this knowledge is limited.

From the reported scientific data, it is clear that WS is a plant with multiple therapeutic potential but clinical trials and more precise studies such as bioavailability and effect of pure compound and its synergistic effect in combination and methods to standardize the percentage composition of active compound(s) in marketed products are the fields yet to be explored.

TABLE 1: LIST OF PATENTS ACCEPTED OR GRANTED ON *WITHANIA SOMINIFERA*

Sl. No.	Title	Applicant	Patent Office	Date of publication
1	A process for the extraction of a fraction mainly containing weakly acidic polysaccharides possessing high adaptogenic activity from the plant *W. somnifera*	Council of Scientific & Industrial Research (CSIR)	India	2/14/98
2	A process for the isolation of polysaccharides having immuno-modulation activity from the plant *W. somnifera*	CSIR	India	4/24/99
3	A process for the isolation of peptides having molecular mass in the range of 110 to 1200 daltons from the plant *W. somnifera*	CSIR	India	11/6/99
4	An improved process for the manufacture of an extract obtained from Ayurvedic medical plant such as Ashwagandha	Ms. J. B Chemicals & Pharmaceuticals Ltd.,	India	01/18/97
5	A method of preparing Ashwagandha Ayurvedic milk chocolate	Girivas Viswanath Shet	India	23/04/1990
6	*Withania somnifera* composition	Natreon Inc.; Indian Herbs Research & Supply Company Ltd.,	USA	28/11/ 2000
7	Formulation for alleviating symptoms associated with arthritis	New England Deaconess Hospital	USA	4/11/ 1997
8	Method of treating musculoskeletal disease and a novel composition there for.	Patwardhan; Bhushan	USA	27/2/ 1996
9	Use of piperine as a bioavailability enhancer	Sabinsa Corporation	USA	26/10/1999
10	Natural Composition for treating bone or joint inflammation	Weisman: Bernard	USA	30/3/ 1999
11	Use of piperine as a bioavailability enhancer	Sabinsa Corporation	USA	28/4/1998
12	Use of piperine to increase the bioavailability of nutritional compounds	Sabinsa Corporation	USA	16/7/1996

*Source: http:// www.indianpatents.org

The current article was an attempt to compile the available information from both traditional and published scientific literatures related to *Withania somnifera*. It will be helpful for the future researchers to get the information in a nut shell and provides opportunities for planning research works related this medicinal plant.

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