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ASHWAGANDHA *(WITHANIA SOMNIFERA)* AS ANTICANCER HERB: AN OVERVIEW.

DR. RASHMI A. JOSHI

Scientist, Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar, Uttarakhand.

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Abstract: *Withania somnifera* commonly called as *Ashwagandha* is a widely used medicinal herb in *Ayurveda*. It is considered to be a Rasayana herb, an adaptogen, and is commonly referred to as 'Indian ginseng'. All the parts of the plant are used in daily tonics and various home remedy recipes to increase health and longevity. Many recent studies have provided evidence for its analgesic, antioxidant, antistress, anti-inflammatory, cardioprotective adaptogenic, antispasmodic, and immunomodulatory and immunostimulant activities. The roots and and leaf extracts contain components that prevent cancer, enhance the effectiveness of cancer therapies, and alleviate the side effects of radiation and chemotherapy. *Ashwagandha* selectively inhibits cancer cells using five signaling pathways and has proven effective against multiple types of cancers. *Ashwagandha* has been found to be effective in cancer treatment as it reduces tumor cell proliferation while increasing overall survival time and also shown to enhance the effectiveness of radiation therapy while potentially mitigating undesirable side effects .It also reduces the side effects of chemotherapeutic agents without interfering with the tumor-reducing actions of the drugs. These effects have been demonstrated in vitro on human cancer cell lines, and *in vivo* on animal subjects, but human trials have been limited. *Ayurveda* has always extolled *Ashwagandha*'s virtues as a wonder herb that improves the body's immunity and vitality. With backing from age-old traditional medicine as well as modern research, *Ashwagandha* presents itself as a herb that holds great promise for integrative cancer care.

Keywords: Chemotherapy, Cancer Treatment, Immunomodulatory, Tumors

Corresponding Author: DR. RASHMI A. JOSHI

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INTRODUCTION

Plants have been a rich source of valuable, cost effective and easily available natural products and plant drugs have been a major source for treatment of diseases for a long time. They have been used in the traditional medicine on the basis of experiences and practice. In developing countries herbal drugs and traditional remedies are relatively more popular because of cultural acceptability and belief that being natural, they are safe and non-toxic.[1] Cancer is probably the most important genetic disease and every year, millions of people are diagnosed with cancer, leading to death in the majority of the cases . The cancer patients have three basic options for treatment. The first which is most conventional utilize chemotherapy, surgery and radiation. Second category includes a wide range of alternative therapies and the third is a combined approach. There are benefits and challenges regardless of which decision is made. The use of surgery, radiation and cancer chemotherapeutic agents still remains the choice of preference of the treatment . Cancer chemotherapeutic agents can often provide temporary relief of symptoms, prolongation of life, and occasionally cures. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects when under-going treatment. Synthesis of modifications of known drug continues as an important aspect of research. However, lots of amount of synthetic work has given relatively small improvements over the prototype drugs. There is a continued need for new prototype-new templates to use in the design of potential chemotherapeutic agents and the natural products are providing such templates. Medicinal plants represent a vast potential resource for anticancer compounds as the chemical constituents present in them can synthesize a variety of structurally diverse bioactive compounds which can reduce or minimize the toxic side effect of chemotherapy and radiation treatment by reinforcing their cancer killing action. The plants interact with stressful environments by physiological adaptation and altering the biochemical profile of plant tissues and producing a spectrum of secondary metabolites. These secondary metabolites are of special interest to scientists because of their unique pharmacophores and medicinal properties. Secondary metabolites like polyphenols, terpenes and alkaloids have been reported to possess antimutagenic and anticancer properties in many studiesThe anticancer activity of medicinal plant derived compounds may result from a number of mechanisms, including effects on cytoskeletal proteins that play a key role in cell division, inhibition of DNA topoisomerase enzymes, antiprotease or antioxidant activity, stimulation of the immune system, etc. Recent studies of tumor inhibiting compound of plant origin have yielded an impressive array of novel structures. Many of these structures are extremely complex, and it is most unlikely that such

compounds would have been synthesized in empirical approaches to new drugs. Modern medicine attributes most cases of cancer to changes in DNA that reduce or eliminate the normal controls over cellular growth, maturation, and programmed cell death.[2-6]

Withania somnifera commonly called as *Ashwgandha* is native to India,but also used among African tribal peoples. *Ayurveda,* the traditional system of medicine practiced in India can be traced back to 6000 BC [7] and for most of these years *Ashwagandha* has been used as a *Rasayana* for its wide ranging health benefits. *Rasayana* is described as an herbal or metallic preparation that promotes a youthful state of physical and mental health and expands happiness. *Ashwagandha* belongs to a sub-group of *Rasayanas* known as *Medhyarasayanas*. *Medhya* typically refers to the mind and its mental and intellectual capacity. Thus, *Medhya Rasayana* like *Ashwagandha*, is used to promote intellect and memory. The cognition promoting effect of *Medhya Rasayana*s is best seen in children with memory deficits, or when memory is compromised following head injury, or a prolonged illness and in old age.[8] It is also known as "*Sattvic Kapha Rasayana*" herb and is mentioned in the ancient Hindu Vedas as an herbal tonic and health food. *Aswagandha* is compared well with *Eleutherococcus senticosus* (Siberian Ginseng) and Panax Ginseng (Chinese / Korean Ginseng) in its adaptogenic properties, and hence it is popularly known as Indian Ginseng. It is also known as "Indian Winter cherry" and is one of the Indian medicinal plants having a remarkable reputation, as a factor of health care, among the indigenous medical practitioners .Several studies over the past few years have indicated that *Ashwagandha* has antiinflammatory, antitumor, antistress, antioxidant, mind-boosting, and rejuvenating properties and medicinally is used as analgesic,aphrodisiac, respiratory stimulant, sedative and tonic. It stimulates the activation of immune system cells, such as lymphocytes ,inhibit inflammation and improve memory in animal experiments. When it is administered with other herbs these actions support the traditional reputation of *Ashwagandha* as a tonic or adaptogen. It also possesses anticonvulsant activity, immunomodulatory effect and cardioprotective effects. Its effective use in the management of nervous disorders is also reported.[9] *In vivo* and *in vitro* studies have been carried out on *Ashwagandha* in various institutions all over the world. The purpose of this paper is to review the literature regarding the anticancerous properties of *Ashwagandha* as several attempts have been taken to elucidate and establish a scientific basis for its use for the treatment of cancer. The different active phytochemicals present in it have been extensively evaluated for their cytotoxic action, their role in improving the haemopiesis, and level of immunity. Studies have also been used to show how this reduce the toxic effects of conventional therapies and improve the well-being of the patient, as an adjuvants to chemotherapy and radiotherapy.

Scientific basis of Cancer and its treatment

A mature human comprises about 10^{15} cells and scores of them divide and differentiate in order to renew organs and tissues, which require cell turnover. However, if the cells do not stop dividing, they can become cancerous. Characteristically, cancer is an uncontrolled proliferation of cells which become structurally abnormal and possess the ability to detach them from a tumor and begin a new lump at a remote site within the host .Almost all types of cancer lead to the progress of tumors, unusual clusters of cells. However, not all tumors are cancerous.[10] Tumors that cannot invade nearest tissues or spread to other parts of the body are called benign tumors. With unusual exceptions, benign tumors do not cause serious diseases and are not life threatening. Malignant tumors are cancerous tumors. Malignant tumors can invade and destroy nearest tissues and organs, and spread to other parts of the body. This spread of cancer cells from one part of the body to an additional distant site is called metastasis. In short, malignant tumors are capable of attack and metastasis, but benign tumors do not have these capabilities In other words cancer is a circumstance which arises while a cell starts disobeying the check mechanisms, which control the rate of cell proliferation and starts dividing in an uncontrolled manner. This leads to the formation of a neoplastic tumor, which is normally benign at this stage, but becomes malignant, when it starts metastasizing, i.e. starts spreading to other tissues. Cancer arises due to either gain of function of a proto-oncogene, and it becomes oncogenic or if there is failure of the function of a tumor suppressor gene .[6] These changes are more likely to occur in people with certain genetic backgrounds and in persons infected by chronic viruses e.g., viral hepatitis may lead to liver cancer, HIV may lead to lymphoma. The ultimate cause, regardless of genetic propensity or viruses that may influence the risk of the cancer, is often exposure to carcinogenic chemicals including those found in nature and/or to radiation including natural cosmic and earthly radiation, coupled with a failure of the immune system to eliminate the cancer cells at an early stage in their multiplication. The immunological weakness might arise years after the exposure to chemicals or radiation. Other factors such as tobacco smoking, alcohol consumption, excess use of caffeine and other drugs,infections from oncogenic virus like cervical papilloma viruses, adenoviruses Karposis sarcoma (HSV) or exposure to asbestos. These are implicated as causal agents of mammalian cancers and a large population of people is often exposed to these agents. Consequently cancer cells continue to divide even in situations in which normal cells will usually wait for a special chemical transduction signal. The tumor cells would ignore such stop signals that are sent out by adjacent tissues. A cancer cell also has the character of immortality even *in vitro* whereas normal cells stop dividing after 50-70 generations and undergoes a programmed cell death called as Apoptosis. Cancer cells continue to grow invading nearby tissues and metastasizing to distant parts of the body. Metastasis is the most lethal aspect of carcinogenesis.[12]

Modern medicine attributes most cases of cancer to changes in DNA that reduce or eliminate the normal controls over cellular growth, maturation, and programmed cell death. Cancer chemotherapy research in conventional medicine is primarily focused on developing analogs of purines, pyrimidines, and various vitamins like folic acid, plant products, and biological products. In conventional medicine, effective anticancer therapy has integrated medical management with surgery and radiation therapy. The development of new cytotoxic and endocrine agents and the introduction of biologic therapy based on recombinant synthesis of interferon and cytokines have helped expand medical management. Not all patients are candidates for cancer therapy because of limitations in available drugs or comorbidity from other medical problems. In addition, not all tumors are responsive to chemotherapy. Although many of the tumors are manageable by various chemotherapeutic agents.

Antitumor drugs fail to cure cancer because they cannot kill 100% of the cancer cells. Even if one cell survives the chemotherapy, that cell can grow very quickly into millions of cells. The result is a relapse of cancer because the body immunity defense does not recognize the remaining cancer cells as foreign cells and thus does not kill them. Cancer cells can hide behind the blood barriers for drugs, such as for blood-brain barrier, and therefore do not get killed. The other mechanisms responsible for the failure of chemotherapy are the metabolism of drugs to inactive form, removal of drugs by binding to plasma proteins, rapid urinary excretion, and the development of resistance in the tumor cells due to an increase in certain critical enzymes that are inhibited by the drug. For example, tumors resistant to methotrexate were found to have high levels of dihydrofolate reductase, a key enzyme.[13-20]

***Withania somnifera* (Ashwagandha)**

The Indian name of *Withania somnifera* is *Ashwagandha* where '*Aashwa*' means horse and '*gandha*' for smell'and now commonly called Asgandh or Asgand, due to the smell that arises from the fresh root. In *Ayurvedic* texts, it is mentioned that this herb imparts the power and sexual strength like that of horse to a man. *Ayurvedic* scholars consider the wild root to be a narcotic and hence probably the specific name '*somnifera*' means 'sleep inducer' which implies its sedative nature .It is used in the Ayurvedic preparations as *Ashwagandhadi churn, Asgandh pak, Ashwagandha Ghrit* as it supports the immune system, acts as an adaptogen, the withanolides present in it produce resistance to chemical, physical and biological stress and as having rejuvenating properties providing the kind of restfully. The traditional physicians prescribe *Ashwagandha* to men and women who suffer from chronic or debilitating illness,in arthritis, rheumatism, fever and against infectious diseases especially in combination with other herbs. In general it is widely used as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy.[21,22] It is used as a powder decoction, fermented drink, mixed with clarified butter, honey or sugar syrup

or as part of medicated oil. The most common form is as an alcoholic extract or capsules of the powdered root. The roots are also cut into small pieces and dried for use and are used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea.[23,24,25]Fruits and seeds are used as diuretic. The fruits of the plant have a milk-coagulating property attributed to the pulp and husk of the berry, which has been used in the preparation of vegetable rennet ferment for cheese.[26] They are also reported to be sedative, emetic and stomachic, blood-purifier and febrifuge, as an alternative, diuretic and bitter tonic in dyspepsia as well as a growth promoter in infants. The shoots and seeds are also used as food and to thicken milk in India. The leaves of the plant are bitter in taste and used as an antihelmantic and combined with astringent and rock salt remove the white spots from the cornea. Bruised leaves and fruits are locally applied to tumors and tubercular glands, carbuncles and ulcers.[27,28,63]

Taxonomy of the plant

Withania somnifera was first described by Carl von Linnaeus as *Physalis somnifera* and then received its current name of M.F.Dunal in 1852. This name is the accepted name of a species in the genus *Withania* of family Solanaceae ,subfamily Solanoideae, tribe *Physaleae* and sub-tribe *Withaninae* of which it is the type genus. *Physalis somnifera* L., *Withania kansuensis* Kuang & A. M. Lu and *Withania microphysalis* Suess.are its synonyms.[29,30] The generic name *Withania* commemorates the celebrated English 'Paleobotanist, 'Henry Thomas Maire Witham' with an orthographic variation of the final 'm' into an 'n' to which the commemorative termination –ia has been added. The specific epithet '*somnifera*' is a compound of two Latin words '*somnus*' meaning sleep and '*fero*' (*ferere*) meaning 'to bear'. Thus the specific epithet alludes to sleep inducing properties of the plant . Among the worldwide list of twenty six species, the genus *Withania* is represented in India by *W. somnifera* and *W. coagulans* and recently a third species *W. ashwagandha* from Indian germplasm using multidisplinary approaches is also confirmed.[31,32]*W. somnifera* is an erect, branched, grayish, stellate-tomentose under-shrub, 30-150 cm high with long tuberous roots. Leaves are simple, petiolate with the leaf blade varying in shape from elliptic-ovate to broadly ovate, entire along margins, acute to obtuse at apex, cuneate or oblique at base, clothed with a persistent grayish tomentum on sides, 4-10 cm long and 2-7 cm broad. Leaves on vegetative shoots are alternate and large and those on floral branches are opposite, arranged somewhat laterally in pairs of one large and one small leaf, bearing in their axil a cymose cluster of 5-25 inconspicuous pale green bisexual flowers. It produces flowers indeterminately round the year with a peak of flowering between March and July. The species exhibits stigma-anther proximity caused by elongation of filaments to cover the bilobed stigmatic surface with dehiscing anthers. High pollen load on the stigma and stiff pollen competition within a flower strongly favours self-pollination . The species has been

reported to show ploidy level variations viz., diploids (2n = 24), tetraploid (2n = 48) and hexaploid (2n = 72) cytotypes besides polysomatomy (2n= 12, 2n= 18, 2n= 24, 2n= 36, 2n= 48 and 2n= 72) with a predominance of 2n= 48 type .[26,32,34].

Cultivation of *Ashwagandha*

This plant due to its medicinal properties is widely grown in countries like Sri Lanka,Nepal, Malaysia, East Indies, and China .The herb grows as a weed in the drier part of India on waste land. A cultivar is being cultivated on a large scale in some parts of central India. The plant of *Ashwagandha* is cultivated in sandy loam or light soils with good drainage. It thrives best in dry climates and often grown as a rain-fed crop with little or no fertilizer application. It is sown in the rainy season and the roots are ready for harvesting in winter .Seeds can be sown directly in the field by broadcasting or the seeds germinated in the nursery and 6 weeks old seedlings are later transplanted into the field with 2 ft (60 cm) spacing between plants and 2 ft (60 cm) spacing between rows.[21]

Chemical Constituents and their anticancerous activities

The chemical components present in *Ashwagandha* are of several groups as steroidal lactones, alkaloids, flavanoids, tannins, saponins etc. Till date more than 12 alkaloids, 40 withanolides and several sitoindosides i.e.,a withanolide containing a glucose molecule at carbon 27 have been isolated and characterized from this species. The concentration of major withanolides usually ranges from 0.001 to 1.5% dry weight.[35-38].Withaferin A (4β, 27-dihydroxyl-1-oxo-5β, 6β-epoxywitha-2-24-dienolide) was the first member of this group of compounds to be isolated and characterized from a South-Asian strain. Some important bioactive molecules isolated from this wonder medicinal plant that have a potential in the drug development programme are Acetyl glucosides as Sitoindosides VII ,Sitoindosides VIII , Glycowithanolide as Sitoindosides IX , Sitoindosides X .The alkaloids are Withanine ,Withananine and the steroidal lactones as Ashwagandhanolide ,Withaferin ,Withaferin A , Withanolie D ,Withanolie E ,Withanone, Withanolide Z , Withanolide B, 7-hydroxywithanolide 3α-methoxy-2, 3-dihydro- 27-deoxywithaferin A, 4β, 17α-dihydroxy-1-1oxo- 5β, 6β-epoxy-22R-witha- 2, 24-dienolide, 4β-dihydroxy-5β, 6β-epoxy- 1-oxo-22R-witha-2, 14-24- , Trienolide ,5, 20α (R)-dihydroxy-6α, 7α-epoxy-1-oxo- (5α) - Witha-2, 24-dienolide are the major constituents . Besides this the aliphatic ester like 2, 3-dihydrowithaferin A-3beta-O-sulfate and Withanolide –WS 2 and aliphatic ketone as Withanolide –WS 1 are also present .[26] Withaferin A and withanolide D are reported to be significant anti-tumor and radiosensitizing withanolides.[39-42]1-oxo-5ß, 6β-epoxy-witha-2-enolide is another constituent of *W. somnifera* reported to reduce the skin carcinoma induced by UV radiations .[43]Withaferin A acts as a mitotic poison arresting the division of the cultured human larynx carcinoma cells at metaphase. It also produces a significant dose dependent

retardation of the growth of Ehrlich ascites carcinoma, sarcoma 180, and sarcoma Black and E 0771 mammary adenocarcinoma.[44]

Ashwagandhanolide ,Withaferin A, Sitoindoside IX,Physagulin D, Withanoside IV and Viscosalactone B inhibit growth and spread of various cancers such as cancers of the breast, lung, colon and central nervous system due to their antiproliferative and antiangiogenic properties. Withaferin A is effective in both androgen-responsive and androgen-refractory prostate cancers. Sitoindosides VII-X and Withaferin A have strong anti-oxidant, antistress, immunomodulatory, anti-inflammatory and antiaging properties. Withanolide D inhibits the metastatic colony formation in the lungs by malignant melanoma.[45,46]

Action of *Ashwagandha* against different types of Cancers

The development of metastases during cancer therapy is also an important factor in the survival of treated patients. On going research on *W. somnifera* suggested that it can be used as an alternative long-term therapy to prevent the spread of cancer cells. The root extracts were tested against vimentin pro-metastatic protein and was found that *Ashwagandha* selectively kills cancer cells. The leaf extract of *Ashwagandha* effectively kills a large variety of human cancer cells including bone, breast, lung, colon, skin, cervical, fibrosarcoma, pancreas, and brain tumors. The selective cancer cell killing activity was assigned to one of its components, Withanone, also called tumor inhibitory factor. The key component of *Ashwagandha* leaf extract and its components kill cancer cells by at least five different pathways, viz. p53 signaling, GM-CFS signaling, death receptor signaling, apoptosis signaling and G2-M DNA damage regulation pathway. Naturally occurring withanone has also been found to bind with and cause inactivation of the TPX2-Aurora A complex, which plays a critical role during mitosis and cytokinesis and is found upregulated in several cancer types. *Ashwagandha* acts as a radiosensitizer i.e.,a drug that makes tumor cells more sensitive to radiation therapy and as a chemotherapeutic agent i.e.,toxic to cells with high proliferation rates.[47-51,58]

Brain Cancer

The constituents of *Ashwagandha* are able to reconstruct neuronal networks and synapses, regenerate axons and dendrites and improve memory deficits. Glioblastoma is the most common and difficult malignant brain tumor to treat. Despite the use of different treatment strategies, including surgery, radiotherapy, and chemotherapy, most patients die within a year of diagnosis. Differentiation therapy in which the malignant cells are treated in such a way so that they stop dividing and resume the process of maturation, is an attractive alternative therapeutic approach. *Ashwagandha* and its components have the potential to induce senescence-like growth arrest and differentiation in glioma cells. *Ashwagandha* extract and its constituents therefore, offer a differentiation-based milder and effective glioma therapy.[47,52,]

Breast Cancer

An open-label prospective non-randomized comparative trial was conducted on 100 patients with breast cancer in all stages undergoing either a combination of chemotherapy with oral *Withania somnifera* or chemotherapy alone. Patients who consumed *Ashwagandha* experienced lower fatigue as compared to those who did not.[54]

Prostate Cancer

Ashwagandha modulates several functionally important classes of genes and molecular signalling mechanisms, which are associated with immune response, inflammation, signal transduction, cell signaling, transcriptional regulation, apoptosis and cell cycle regulation. This makes it an effective chemopreventive agent relevant to prostate cancer progression.[55]

Renal cancer

Ashwagandha has been found to induce cell death in Caki human renal cells by down-regulating the STAT3 signalling pathway, inhibiting JAK2 phosphorylation and suppressing the expression of harmful proteins.[56]

Skin Cancer

A study was conducted on Swiss Albino mice with induced skin cancer. Treatment with *Ashwagandha* root extract resulted in a significant decrease in incidence and average number of skin lesions; biochemical parameters were also returned to near normal. The researchers inferred that the antioxidant/free radical-scavenging constituents and the anti-inflammatory and immunomodulatory properties of *Ashwagandha* extract might be responsible for its chemopreventive action.[57]

Pharmacological studies

Many pharmacological studies have been conducted to investigate the properties of *Ashwagandha* in an attempt to authenticate its use as a anticancerous herb. Several studies have examined the antitumor and radio sensitizing effect of *W. somnifera*. The anticancerous activity of *W. somnifera* can be attributed to its active phytochemicals present in it as well as its action in the form of different extracts prepared from the parts of the plant. Chemo-preventive activity is attributed partly to the antioxidant/free radical scavenging activity of the ex-cytoskeleton architecture alteration by covalently binding annexin II ,[59,] anti-tumor capacity by inhibition of proteasomal chymotrypsin-like activity[60] and apoptosis induction through the inhibition of protein kinase C or activation of caspase-3 [61,62] have also been explored. The withaferin A-mediated suppression of breast cancer cell viability correlated with apoptosis

induction characterized by DNA condensation, cytoplasmic histone–associatedDNA fragmentation, and cleavage of poly-(ADP-ribose)-polymerase.[46]

Anticancerous activity

For the establishment of anticancer potential of *W. somnifera* many types of studies have been conducted for their cytotoxic activities, antitumor effect as well as its chemopreventive and immunomodulatory effects .

The methanolic extract of *W. somnifera* has been used in stem cell proliferation and it inhibited growth of breast, lung, central nervous system and colon cancer cell lines by decreasing their viability in doze dependent manner and therefore holds promise as a chemotherapeutic agent .[45,64] Administration of 75% methanolic extract of *W. somnifera* was found to significantly increase the total white blood cell (WBC) count in normal Balb/c mice and reduce the leucopenia induced by a sublethal dose of gamma radiation. Treatment with *W. somnifera* was found to significantly increase the bone marrow cellularity in mice. [65] Its extract also inhibited 20-methylcholanthrene-induced sarcoma development in mice at a dose of 20 mg/day and was also found to reduce two-stage skin carcinogenesis induced by DMBA and croton oil.[66,67] *W. somnifera* was found to significantly reduce leucopenia induced by cyclophosphamide (CTX) treatment. *Withania* extract increased the number of alpha-esterase positive cells in the bone marrow of CTX plus *W. somnifera*-treated animals, compared with the CTX-treated group[68].Withaferin A, isolated from the roots of *W. somnifera,* was found effective in Ehrlich ascitis carcinoma when given at a dose of 30 mg/kg along with radiation therapy.[68-70] It reduced survival of V79 cells in a dose-dependent manner. LD_{50} for survival was 16 mM. One-hour treatment with a non-toxic dose of 2.1 mM before irradiation significantly enhanced cell killing, giving a sensitizer enhancement ratio (SER) of 1.5 for 37% survival and 1.4 for 10% survival.[71]

Antitumor activity

The alcoholic extract of dried roots of *W. somnifera* and Withaferin A showed significant antitumor and radio-sensitizing effects in experimental tumors *in vivo* without any noticeable systemic toxicity. *W. somnifera* was injected at a dose of 500 mg/kg to tumor-bearing mice (tumor size: 50 ± 5 mm^3) for 10 days with one local exposure of radiation therapy followed by hyperthermia. It significantly increased the tumor cure rate, produced a delay in the growth of partially responding tumors, and increased animal survival.This study concluded that *W. somnifera*, in addition to having a tumor-inhibitory effect, also acts as a radio sensitizer. The mechanism of action, however, is not clear but the studies indicate that *W. somnifera* could prove to be a good natural source of a potent and relatively safe radio-sensitizer and chemotherapeutic agent [40,41].*W. somnifera* was also found to enhance the levels of interferon gamma (IFN-gamma) , Interleukin-2 (IL-2) and granulocyte macrophage colony stimulating

factor (GM-CSF) in normal Balb/c mice. The lowered levels of IFN-gamma, IL-2 and GM-CSF after treatment with CTX was reversed by the administration of *W. somnifera* extract. The extract lowered the levels of tumor necrosis factor alpha (TNF alpha) production. Administration of bone marrow cells from donor mice treated with the extract increased the spleen nodular colonies in irradiated mice compared with those treated with normal bone marrow cells. The number of nodular colonies increased significantly after continuous treatment with *W. somnifera*. [72] *W. somnifera* was also found to increase the neutrophil count in mice with paclitaxel-induced neutropenia[73].The extract was evaluated for its antitumor effect in urethane-induced lung adenomas in adult male albino mice.[77] Simultaneous administration of its ethanolic extract 200 mg/kg daily orally was given for seven months and urethane (125 mg/kg without food biweekly for seven months) reduced tumor incidence significantly. The histological appearance of the lungs of animals protected by *W. somnifera* was similar to those observed in the lungs of control animals. No pathological evidence of any neoplastic change was observed in the brain, stomach, kidneys, heart, spleen, or testes of any treated or control animals. In addition to providing protection from carcinogenic effects, this treatment also reversed the adverse effects of urethane on total leukocyte count, lymphocyte count, body weight, and mortality. The growth inhibitory effect of *W. somnifera* was also observed in Sarcoma 180 (S-180), a transplantable mouse tumor.[39] Ethanol extract of this plant root (400 mg/kg and up, daily for 15 days) after intra-dermal inoculation of $5x10^5$ cells of S-180 in BALB/c mice produced complete regression of tumor after the initial growth. A 55% complete regression was obtained at 1000 mg/kg; however, it was a lethal dose in some cases. *W. somnifera* was also found to act as a radio- and heat sensitizer in mouse S- 180 and in Ehrlich ascites carcinoma.Antitumor and radiosensitizing effects of Withaferin were also seen in mouse Ehrlich ascites carcinoma in vivo.[80] Withaferin A gave a radiosensitizer ratio of 1:5 for in vitro cell killing of V79 Chinese hamster cell at a non-toxic concentration of about 2 mM/L.*Withamia* roots caused the inhibitory effect of about 49% on colony forming efficiency of CHO cells. It inhibits the cell growth and prevents the cell attachment and induces long term growth inhibition of CHO cells which was dependent on the cell density and duration of *Ashwagandha* exposure. [39,68,79]*Ashwagandha* in the treatment of fibroid tumors of uterus showed reduction of uterine bleeding tendencies and disappearance of fibroids after long treatment.[11]

Chemopreventive and Immunomodulatory properties

Chemopreventive activity is attributed partly to the antioxidant/free radical scavenging activity of the ex-cytoskeleton architecture alteration by covalently binding annexin II , anti-tumor capacity by inhibition of proteasomal chymotrypsin-like activity , and apoptosis induction through the inhibition of protein kinase C or activation of caspase-3.[59-62] *W. somnifera* extract was also found effective as a chemopreventive agent as it protected against 20-

methylcholanthrene-induced fibrosarcoma tumors in Swiss albino mice. A single subcutaneous injection of 200 mg of 20-methylcholanthrene in 0.1 ml of dimethylsulphoxide into the thigh region of mice produced a high incidence (96%) of tumors. Oral treatment of animals with 400 mg/ kg body weight of *W. somnifera* extract (1 week before injecting 20-methylcholanthrene and continuing until 15 weeks thereafter) significantly reduced the tumor incidence and tumor volume and enhanced the survival of the mice, compared with the untreated 20-methylcholanthrene-injected mice. The occurrence of tumors was also delayed in the treatment group. Liver biochemical parameters revealed a significant modulation of reduced glutathione, lipid peroxides, glutathione-S-transferase, catalase, and superoxide dismutase in extract-treated mice compared with 20-methylcholanthrene-injected mice. These authors suggested that the mechanism of chemopreventive activity of *Withania somnifera* extract may be due to its antioxidant and detoxifying properties.[74,78]

Glycowithanolides and a mixture of sitoindosides IX and X isolated from *W. somnifera* were evaluated for their immunomodulatory and central nervous system effects[75]. Administrated 50-200 mg/kg orally both compounds also produced significant antistress activity in albino mice and rats. They also augmented learning, acquisition and memory retention in both young and old rats. Root extract of *W. somnifera* was tested for immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin or prednisolone . Significant increase in hemoglobin concentration, red blood cell count, white blood cell count, platelet count and body weight were observed in *W. somnifera* treated mice compared to controls. A significant increase in hemolytic antibody responses toward human erythrocytes which indicated immunostimulatory activity was also reported.[45]The effect of *Ashwagandha* 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.[5,40,41,81]

Conclusion

Ashwagandha plays a significant role in the treatment of cancer and most new clinical applications of its secondary metabolites and their derivatives have been applied towards fighting cancer. The available scientific data support the conclusion that *Ashwagandha* is found to be very useful in experimental carcinogenesis in the crude form as well as its phytochemicals are also effective in the treatment .It can be used as an adjunct to cancer chemotherapy or radiotherapy. Besides having an anti-cancer effect it will also reduce the side effects of anti-

cancer agents, which invariably reduce immunity and quality of life. It also acts as an immunomodulator and hence can enhance life span of cancer patients, where lowered immunity states of the patient are the cause of concern. The research and studies of *Ashwagandha'*s activities in the inhibition and reduction of tumour growth have shown encouraging evidence that this remarkable herb may prove to be extremely effective in the treatment of tumor type diseases including cancer .[33,53,82-84] It also improves the white cell count and function, which are depleted in the chemotherapeutic treatment of cancer.[11] Some practitioners are also using *Ashwagandha* in all forms of cancer including prostate and lung cancers, especially in last stages, giving the patients lot of health benefits. Some patients of lung cancer who have refused modern therapy and recovered clinically and radiologically with the therapy of *Ashwagandha.* These findings clearly indicate that the traditional use of *Ashwagandha* has a logical and scientific basis. This knowledge in turn will assist oncologists who plan to use the *Ashwagandha* as 'synergizers with conventional chemotherapy or radiation therapy. But still the large scale clinical studies are needed to prove the clinical efficacy of this herb ,specially for cancer. There is further need to investigate in depth the active constituents present in *Ashwagandha*,their isolation ,characterization and their anticancerous activities. The interface between cell biology, *in vitro* assays and structural chemistry will be the best way forward to obtain valuable leads. An interaction between traditional medicine and modern biotechnological tools is to be established towards new drug development for cancer.

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