Review Article

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Withania Somnifera: A New Approach To Cancer

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ABSTRACT

Cancer is a hyper proliferative disorder characterized by the abnormal proliferation of cells that invades into the adjacent tissues and causes the destruction. Many cancers are hereditary, other arise from the internal and external environmental factor. Various treatment modalities available for cancer are Surgery, Radiation, Chemotherapy and targeted therapy. Inspite of major advances in drug discovery, research is still undergoing to understand complex biology of cancer with new therapeutics available. A popular ayurvedic herb Withania Somnifera (WS) also known as Indian Winter Cherry and Indian Ginseng is used as a traditional medicine for various cancers. The results of the studies carried out on WS and its chemical components like Withaferin A(WA), Withanolide D (WithaD) etc, describe that it is effective in prevention and treatment of different kinds of cancer like colon, blood, lung, skin, breast, renal, fibrosarcoma, prostate, pancreatic, renal, malignant melanoma, osteosarcoma by preventing proliferation and progression of cancer cells, alteration in hematological and biochemical parameters, modulation of cell cycle markers etc. Withania somnifera can be given alone or in combination with synthetic drugs. Identification of novel natural anticancer compound is highly demanding for prevention and treatment of cancer. However, multicentric long term clinical studies should be carried out to provide complementary and alternative therapy.

Keywords: *Ashwagandha, Withania Somnifera, Oral cancer, Ayurvedic Herb.*

Introduction

Cancer is dreaded and life threating disorder characterized by proliferation, invasion, transformation, dysregulation of apoptosis and metastasis.[1] It defines a large group of disease originating from uncontrolled cell division in any part of body. Many cancers are hereditary, other arise from the internal and external environmental factor. Various treatment modalities available for cancer are Surgery, Radiation, Chemotherapy and targeted therapy. [2] Chemotherapy and Radiotherapy are harmful causing damage to adjacent healthy cells with devastating side effects affecting quality of patient's life.[3]. Inspite of major advances in drug discovery, research is still undergoing to understand complex biology of cancer with new therapeutics available[4].For novel and safe therapies, a great emphasis is given towards complementary and alternative approach i.e. Ayurveda. It is a traditional medicine successful from ancient times in using natural drug for prevention and suppression of various cancer by various pathways such as inhibition of cell proliferation, stimulation of apoptosis or inhibition of free radicals etc.[5,6,7]

A popular ayurvedic herb Withania Somnifera (WS) also known as Indian Winter Cherry and Indian Ginseng is used as a traditional medicine for various cancers. In Sanskrit it is called as Ashwagandha where 'Aashwa' means horse and 'gandha' is smell.[1,6,8] It is perennial plant belonging to Solanaceae family.[9] There are 23 known species of which WS and Withania Coagulans have medicinal benefits.[4]

Ashwagandha is erect, branched shrub with long roots .Leaves are simle,petiolate varying in shape from oval, elongated, acute to obtuse at apex and oblique at base. Flowers are produced intermittently with a ripe orange-red fruit.[1,6] It is cultivated in drier regions of Manasa, Neemuch, Mandsaur of Madhya Pradesh, Punjab, and Rajasthan.[1]

The chemical constituents in the roots of WS are categorized in several groups such as steroidal lactones (Withanolides, Withaferins), alkaloids (Isopelletienin, Anaferine), flavanoids, tannins, saponins and several sitoindosides. WS is also rich in iron. The alkaloids such as Withanine, Withananine and the steroidal lactones as Ashwagandhanolide, Withaferin, Withaferin A (WA), Withanolide D (WithaD), Withanolide E, Withanone, Withanolide Z, Withanolide B, 7-hydroxywithanolide 3α-methoxy-2, 3-dihydro- 27deoxywithaferin A, 4β, 17α-dihydroxy-1-1oxo- 5β, 6β-epoxy-22R-witha- 2, 24-dienolide, 4βdihydroxy-5β, 6β-epoxy- 1-oxo-22R-witha-2, 14-24- , Trienolide ,5, 20α (R)-dihydroxy-6α, 7αepoxy-1-oxo- (5α) - Witha-2, 24-dienolide are the major constituents. WA and WithaD possess significant anti-tumor and radiosensitizing properties. [1,6,10]

The leaves contain steroidal lactones commonly called as withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, with a six memebered lactone ring. Ashwagandhanolide, WA, Sitoindoside IX, Physagulin D, Withanoside IV and Viscosalactone B have effects on

various cancers such as cancers of the breast, lung, colon and central nervous system due to their antiproliferative and antiangiogenic properties.[6,10]

This review focuses on the cultural and clinical studies conducted on WS, to evaluate its efficacy on various cancers, for better understanding of its mechanism and providing a new way to treat cancer.

Anticancer properties of Withania somnifera

WS has anticancer properties against various cancers such as colon, blood, lung, skin, renal, prostate, pancreatic, fibrosarcoma, malignant melanoma, cervical, osteosarcoma, breast, head and neck squamous cell carcinoma. Various invitro, and animal studies have been conducted on different parts of Ashwagandha like roots, stems, and leaves to check its anticancer activity.

1] Colon Cancer: WS shows significant alteration in levels of Leucocytes, Lymphocytes, Neutrophils, Immune complexes and Immunoglobulin levels and also considerable reduction in polyethylene glycol (PEG) indices in azoxymethane induced colon cancer of swiss albino mice.[11].In one more animal study, WS showed decreased activity of TCA cycle enzymes and electron transport chain complexes.[12] Ethanolic extract of roots, leaves and stems of WS were evaluated for cytotoxicity against five human cancer cell line i.e PC-3,DU-145(prostate),HCT-15(colon),A-549(lung) and IMR-32 (neuroblastomas) and showed high cytotoxicity to this human cell lines.[13] WA, biologically active compound derived from WS, inhibits Notch-1/Akt/NF-kB/Bcl-2 signaling pathways in three colon cancer cell lines(HCT-116,SW-480 and SW-620) and it also not only inhibits the proliferation of HCT116 cells but also suppressed the tumor growth in vivo by blocking STAT3 signaling pathways.[14,8] WA causes cell cycle arrest at G2/M phase on colon cancer cell lines HCT116 and SW480 by degradation of Spindle Assembly Check(SAC) proteins like Mad2 and Cdc20 leading in delayed mitotic activity and apoptosis of cells.[15]

2] Blood Cancer: Various studies have been performed on human leukemia HL-60 cells and other cells like Molt-4, PC-3, DU 145, HuT-78 and HeLa cells where WA induces early ROS generation and mitochondrial dysfunction triggers the apoptosis pathway. It also increases activity of caspase-8 and caspase-9 leading to activation of extrinsic and intrinsic pathway and thus WA may have possible anticancer property if present in dietary supplement of WS.[16] WithaD is a pure herbal compound purified from the ancient medicinal plant WS which can induces apoptosis in both myeloid and lymphoid cells(invitro) and in primary cells arised from leukemia patients (invivo) by activating N-SMase 2 and assembling ceramide content which activate MKK group of proteins, phosphorylation of the JNK and p38MAPK.[17] L-asparaginase obtained from Withania somnifera L. had anticancer activity against acute lymphoblastic leukemia[1].In another study, growth inhibitory effect of methanolic leaf extract of WS and Withanolide, on promyelocytic leukemia cells (HL-60) showed apoptosis in cell by decrease in the Bcl-2/Bax ratio, leading to up regulation of mitochondrial signaling through Bax resulting in cytochrome c release and activation of caspase 3,8 and 9,thus caspase playing an important role in apoptosis pathway of cells.[18] Dimethyl sulfoxide extract obtained from the roots of WS have significant cytotoxic and cytostatic effect and can induce ICD(Immunogenic cell death) by intracellular Ca2+ accumulation and the production of reactive oxygen species in human T-lymphoblastoid cell line.[19]

3] Lung Cancer: Senthilnathan P. et al demonstrated use of synthetic anticancer drug like paclitaxel in combination with WS to treat the benzo pyrene-induced lung cancer in mice and considered it as potent chemotherapeutics agents.[20] Three compounds isolated from WS as chlorinated steroidal lactone, a diepoxy withanolide and WA displayed growth inhibitory and cytotoxic activity against lung cancer cell line(NCI-H460). Among these WA was found to be more effective.[21] WA a bioactive lactone; revealed dose dependent cytotoxicity and caused oxidative damage to non-small cell lung cancer (NSCLC) cells by involvement of ROS with minimum damage to normal cells.[22] WS shows anticancer activity against urethane induced lung-adenomas in adult male albino mice by inducing a state of non-specific increase in resistance (SNIR), reversing the hematological parameters like total count and improving the immune status.[23]

4] Skin Cancer: In carcinogen-induced forestomach and skin tumorigensis in Swiss albino mouse model, dietary administration of WS roots on hepatic phase I, phase II, and antioxidant properties, revealed that WS inhibited phase I, activated phase II and antioxidant enzymes with minimal side effects acting as preventive property against tumorigensis in mice.[24] 1-oxo-5beta, 6beta-epoxy-witha-2-enolide a chemical constituent was obtained from roots of WS, where it proved to be a successful agent in preventing the incidence of skin carcinoma caused by UV B radiation.[25] In another study, chemopreventive effect of WS hydroalcoholic root extract (WSRE) on 7, 12-dimethylbenz[a] anthracene (DMBA)-induced skin cancer was investigated on Swiss albino mice. WRSE showed potential chemopreventive activity, contributed by anti-inflammatory and immunomodulatory

properties.[26] Furthermore studies were conducted on skin carcinogenesis mouse model by using WA. Up-regulation of ACC1 (acetyl-CoA carboxylase 1) by WA can act as chemopreventive and therapeutic agent in a chemically-induced skin carcinogenesis mouse model by inhibiting cell proliferation rather than inducing cell death.[27]. WA represses the 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced cell transformation and cell proliferation in Murine skin epidermal JB6 Cl-41 P+ cells. Administration of WA also reverses the IDH1 activity and mitochondrial function, thus act as a chemopreventive agent.[28]

5] Renal Cancer: Renal carcinoma is radio resistant malignancy and 10th most common cancers in the U.S. Novel radiosensitizer should be identified which help in improving therapeutic effect of radiation on cancer cells. In one such study, combination of WA and radiation together, compared to WA or radiation administered alone, revealed that WA boosts radiation-induced apoptosis in Caki cells (renal carcinoma cells).Thus WA may act as radiosensitizer for better therapy.[29]. WA induces apoptosis in Caki cells by downregulation of STAT3 signaling pathway regulating genes such as bcl-xL, Bcl-2, Cyclin D, and survivin and is also associated with decrease in Janus-activated kinase 2(JAK2) activity.[30] WA induces Endoplasmic reticulum (ER) stress mediated apoptosis in Caki cells by splicing of XBP1 mRNA, phosphorylation of eIF-2a (eukaryotic initiation factor-2α), inhibition of caspase-4 activity by z-LEVD-fmk, elevated expression of GRP78(glucose-regulated protein) and CAAT/enhancer-binding protein homologous protein (CHOP).[31] Further studies on renal carcinoma was carried out using TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) protein derived from immune cells that functions as a ligand to induce cell death by apoptosis. TRAIL binding to death receptors (DR4 and DR5) through recruitment of FAS-associated protein and caspase-8 and it targets cancer cell due to higher expression of death receptors than normal cells. Withanolide, WA (2a) derived from WS were used as sensitizers of RCC (renal carcinoma cells) to TRAIL-mediated apoptosis and also there was identification of the 17β-hydroxywithanolide (17-BHW), withanolide E (1). It revealed that withanolides 1, 2a, 2c, and 3−36 have better ability to sensitize TRAIL-mediated apoptosis in RCC. Thus Withanolide 1 was able to sensitize TRAIL to induce apoptosis of RCC and Withanolide 3 has potent and selective cytotoxic activity to other cancer cells.[32]

6] Prostate Cancer: Prostate cancer (CaP) is the third leading cause of cancer related death in men in western countries. Natural compounds like WA can inhibit carcinogenesis and help in preventing cancer. It produces irreversible arrest at G2/M phase of the cell cycle in both CaP cell lines (PC3 and DU145) and accompanied by upregulation of phosphorylated Wee1, histone H3, p21 with downregulation of cyclins (E2, A, and B1) and phosphorylated Cdc2 (Tyr15). Thus WA can be used as therapeutic agent in prostate cancer.[33] WS regulates several classes of genes in PC-3 such as downregulation of IL-6, IL-1b, chemokine IL-8, Hsp70 and STAT-2, with a corresponding upregulation of p38 MAPK, PI3K, caspase 6, Cyclin D and c-myc.[34] DNA microarray analysis showed that WA significantly increases mRNA levels of c-Fos and 11heat-shockproteins (HSPs) and also cause distortion of vimentin cytoskeleton in prostate cancer. distortion of the vimentin cytoskeleton plays crucial role in WA-mediated apoptosis in androgen-independent PC-3 and DU-145.[35] WA produces apoptosis by up-regulation of Par-4 gene (prostate apoptosis response-4 gene) expression in androgen-refractory prostate cancer cells but not in androgen responsive (WT AR or AR mutant) prostate cancer cells and normal prostate epithelial cells.[36]

7] Pancreatic Cancer: Pancreatic cancer (PC) is the fourth most cancer related death in US with worst prognoses among malignant tumors. Cancer cells show alterations in protein synthesis that regulate endoplasmic reticulum (ER) homeostasis and degradation by ubiquitin-proteasome system (UPS) and autophagy. In one of the study on PC, WA showed significantly increased in autophagosomes, but blocked the degradation of autophagic cargo by inhibiting soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE)-mediated fusion of autophagosomes and lysosomes in PC cells.[37] In another study, *WS* was given in combination with synthetic anticancer drug such as oxaliplatin on PC cells lines like Panc-1, MIAPaCa-2, and SW199 *in vitro* and *in vivo. WS* promoted growth suppression and apoptosis through ROS-mediated mitochondrial dysfunction and by inhibition of the PI3K/AKT pathway whereas in *invivo*, showed potential anti-tumor effects compared to single factor administered, without much side effects and toxicity.[38] Further studies of WA on PC cell lines like Panc-1, MiaPaca2 and BxPc3 and in vivo pancreatic cancer xenograft, exhibited cytotoxicity. [39] Witha-D promotes cell-cycle arrest at the G2/M phase via inactivation of the Akt/Gsk3β kinase cascade and with simultaneous dysregulation of the Wnt/β-catenin pathway leading to apoptosis in pancreatic ductal adenocarcinoma cells lines. Thus Witha-D has apoptotic effects in pancreatic adenocarcinoma, and can be used as advanced drug in treatment of various diseases.[40]

8] Fibrosarcoma: Studies on fibrosarcoma using WS revealed that, WS inhibits 20-methylcholanthrene induced sarcoma development and the lipid peroxide formation, but increases the GSH and GST level as compared to control

group.[41] Hydroalcoholic WS roots extract concentration against 20-methylcholanthrene induced fibrosarcoma tumors in Swiss albino mice shows alteration in liver biochemical parameters such as reduction of glutathione, lipid peroxides, glutathione-S-transferase, catalase and superoxide dismutase due to antioxidant and detoxifying properties.[42] Anti-tumor effect of various extracts of the plants such as WS, Psidium guajava L. (Myrtaceae), Laurus nobilis L. (Lauraceae) and Salvia fruticosa M. (Labiatae) on the fibrosarcoma (L929sA) cells, and human breast cancer cells (MDA-MB231 and MCF7) were evaluated. Among all these extracts WS showed inhibitory activity of nuclear transcription factor NFkappaB, leading to apoptosis and immunomodulation.[43]

9] Melanoma: Melanoma is the most aggressive disease and various treatment modalities have been available but none has proven its efficacy. WA induces apoptosis in different human melanoma cell lines by triggering ROS generation, down regulation of Bcl-2 and also associated with Bax mitochondrial translocation, release of cytochrome c into the cytosol, caspase 9 and 3 activation.[44] In another study, WA revealed increase in the tumor response in B16F1 melanoma cells induced in C57BL mice during repeated HT(hyperthermia) treatment by not completely inhibiting the development of thermotolerance but reducing its magnitude and recovery time.[45] Crude water extract of WS roots showed cytotoxic effect on human malignant melanoma A375 cells, thus suggesting a potential chemotherapeutic effect of it.[46]

10] Cervical Cancer: Cervical cancer is the second leading cause of female cancer deaths worldwide most commonly associated with HPV in 99.5% cases. WA has antiproliferative activity on human cervical cancer cells in vitro and in vivo. In vitro WA inhibited the cells by downregulation of HPV E6 and E7 oncoproteins, causing cell cycle arrest at G2/M phase, with alteration in levels of p53-mediated apoptotic markers such as Bcl2, Bax, caspase-3. In vivo, WA showed decrease in 70% of the tumor volume, thus acting as a therapeutic agent against cervical cancer, either as single drug or in combination.[47] Three different extracts were prepared from WS, Ocimum sanctum and Azadirachta indica, where WS showed both apoptosis as well as reversal of hypermethylation of RARβ2 gene on HeLa cell line.[48] WA can also retards TGF-β-induced invasion and metastasis with downregulation of expression of MMP-9 through the Akt pathway in Caski and SK-Hep-1 cells.[49]

11] Osteosarcoma: WA can be used as a beneficial agent in various osteosarcoma cell lines by producing apoptosis via ROS production and distortion of mitochondrial membrane potential in dose-dependent manner and caspase-3 activation.[50] WA can also have antiproliferative activity on U2OS and MG-63 cell lines by inducing cell cycle arrest at the G2/M phase, associated with hindrance of cyclin B1, cyclin A, Cdk2 and p-Cdc2 (Tyr15) expression and increased level of p-Chk1 (Ser345) and p-Chk2 (Thr68).[51]

12] Breast Cancer: WA and WS Root Extract standardized (sWRE) inhibited breast cancer cells by inhibition of cell motility via distorting vimentin morphology and Epithelial to Mesenchymal Transition *in both human xenograft and mouse mammary carcinoma model.*[52] WA also possess anticancer activity by retarding EMT in MCF-10A cells, and inhibits growth of tumor cells in mammary cancer by squashing vimentin protein expression.[53] WA induced apoptosis in MCF-7 (estrogen responsive) and SUM159 (triple negative), by downregulation of extracellular signal-regulated kinase (ERK), activation of p38 MAPK (mitogen-activated protein kinases) and also induction of long and short forms of Mcl-1(myeloid cell leukemia-1).[54] WA shows inhibitory activity on MDA-MB-231 and MCF-7 by activating Notch2 and Notch4 signaling pathway and also by repression of XIAP, Survivin, and cIAP-2. Whereas invivo WA-mediated interference in MDA-MB-231 xenograft by suppression of Survivin protein only.[55,56] WA reveals less viability of cells in cell lines such as MCF-7 and MDA-MB-231 by decreasing cellular proliferation and increasing apoptosis along with excessive decrease in protein levels of Bim and its transcriptional regulator FOXO3a. Invivo administration of WA in MDA-MB-231 xenograft resulted in reduced growth of tumor by inducing apoptosis, linked to reduced cellular proliferation and PCNA expression.[57] Alcoholic extract of stems of WS revealed potential anticancer activity as compared to aqueous extract.[58] In one of the clinical study, WS extract showed improvement in human breast cancer patients by refining chemotherapy-induced fatigue and quality of life (QoL).[59]

13] Brain Cancer: Ashwagandha water extract (ASH-WEX) have anti-proliferative activity on neuroblastoma cell line such as IMR-32, SH-SY5Y and Neuro-2a by inducing apoptosis through upregulation of NCAM (neural cell adhesion molecule), modulation of cell cycle markers and increasing Akt-P expression leading to cell cycle arrest at G0/G1 phase.[60] Methanolic extract of roots of WS encouraged dendrites formation and regenerated the neuronal network by increasing expression of dendritic markers in human neuroblastoma cells.[61]

14] Head and Neck Squamous Cell Carcinoma: Recently the study was conducted on HNSCC cells like i.e MC3

and HN22 by using methanolic extract of Ashwagandha (MEAG), which induced apoptosis by Bim and DR5, caspase8, t-Bid signaling.[62]

Discussion

Cancer is hyper proliferative disorder characterized by the abnormal proliferation of cells that invades into the adjacent tissues and causes the destruction[4]. It is one of the major threats of modern life with increasing incidence in 21st century and considered as the second most common cause of death after myocardial infarction.[63] It occurs due to disturbance in two genes i.e., oncogenes responsible for the growth of cancer cells, and tumor suppressor genes prevents cancer from developing. Though it is not possible to evaluate the specific cause for specific cancer, other contributing factors such as tobacco use, alcohol, environmental pollutant, infectious agents, and life styles can increase the risk of cancer.[64]

Charaka and Sushruta, well known Ayurvedic Literature describe 'cancer' as inflammatory or noninflammatory swelling and mentions as 'Granthi' (minor neoplasm) or 'Arbuda' (major neoplasm). It classifies three body-control system i.e. Nervous system (Vata) ,Venous system (Pitta) and arterial system (Kapha) for normal functioning of body. In benign neoplasm, one or two system are affected but body tries to reconcile it, while in malignant neoplasm all three system are affected, leading to progression of cancer.[5]

Modern science has done major research in understanding cancer and its molecular basis, but the knowledge about how to prevent or treat cancer is still lagging behind.[65] Antitumor drugs fail to cure cancer because they cannot kill all the cells completely and even if one cell remains, it can multiply and cause increase risk of cancer. Chemotherapy and radiation therapy has major disadvantage that, along with cancer cells the normal healthy cells are also killed and thus immunity of person is hampered and there can be relapse of cancer because the body immunity defense does not recognize the remaining cancer cells as foreign cells and thus does not kill them. 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. The medicinal plants contain chemical constituents of therapeutic value which produce physiological action on the human body. The anticancer activity of medicinal plant derived compounds have 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.[6]

One of popular Indian medicinal plant used for over 3000 years in Ayurvedic medicine to treat diverse range of diseases is "Ashwagandha (Withania somnifera)". It is a member of the class of herbs called 'rasayana' which is described as an herbal or metallic preparation that promotes a youthful state of physical and mental health and expands happiness. Ashwagandha also known as "Sattvic Kapha Rasayana" herb and is mentioned in the ancient Hindu Vedas as an herbal tonic and health food.[9,6]

Ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, hemopoetic, immunomodulatory, anticonvulsant and rejuvenating properties and appears to have benefit on the endocrine, cardiopulmonary, and central nervous systems. It also stimulates the activation of immune system cells, such as lymphocytes, thus boosting the immunity, also inhibit inflammation and improve memory in animal experiments.[6,10] It is used as an ingredient in many musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy.[1]

The results of the above studies describes that WS and its chemical components like WA, Witha D etc are effective in prevention and treatment of different kinds of cancer like colon, blood, lung, skin, breast, renal, fibrosarcoma, prostate, pancreatic, renal, malignant melanoma, osteosarcoma by preventing proliferation of cancer cells, delays the progression of tumor, alteration in hematological and biochemical parameters, modulation of cell cycle markers etc. Withania somnifera can be given alone or in combination with synthetic drugs. Sitoindosides VII-X and WA have strong anti-oxidant, antistress, immunomodulatory, anti-inflammatory and antiaging properties. It can be used as alternative long-term therapy to prevent spread of cancer.

Conclusion

Identification of novel natural anticancer compound is highly demanding for prevention and treatment of cancer. WS is a plant used from ancient times of Indian medicine, which has potential anticancer activity. Many invitro studies are carried out using this plant extract but long term clinical studies must be conducted to provide complementary and alternative therapy.

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