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Role of Withaferin A in the management of breast cancer: A comprehensive review

Review Article

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Abstract

Background: Breast cancer is one of the leading causes for cancer mortality. The conventional treatments are being reported for many side effects which affects quality of life of a patient. Novel therapeutic and preventative strategies from the medicinal herbs are needed to reduce suffering, disease free survival, and mortality from breast cancer. *Withania somnifera* L. Dunal (Indian winter cherry or *Ashwagandha*) from the *Solanaceae* family is an appealing medicinal plant widely investigated for its breast cancer potential. Ayurveda treaties explained various formulations using root of *Ashwagandha*. Modern science explained uses of root and leaf in extract forms. Withaferin A is a promising anticancer withanolides. This review is based on in-vitro researches of Withaferin A on breast cancer cell lines like MCF-7 cells, MDA-MB-231, SUM159, MDA-MB-468, SUM149,SUM159, 231MFP supported by its mechanism, in-vivo studies and clinical records. Material and methods: This review is based on various preclinical researches related to breast cancer. Moreover, this review represents the effect of Withaferin A on cancer cell. Various articles including studies and description of *Ashwagandha* were reviewed using databases namely Google Scholar, PubMed, Web of Science, Scopus. Result:Withaferin A significantly arrests the growth of many breast cancer cell in vivo and in vitro. Conclusion: *Ashwagandha* is a commonly available, cost effective natural medicine,possess anti-cancer potential. It can serves a add on treatment strategy for breast cancer management, chemoprevention, tumor suppression.

Keywords: Anticancer, *Ashwagandha, Ayurveda,* Breast cancer, ER+ve, PR+ve, TNBC, Withania somnifera, Withaferin-A.

Introduction

According to World Health Organization fact sheet 2020 on Non-communicable diseases; reported mortality on cancer is 9.3 million. (1) Cancer is one of the leading causes of death globally. In 2020; approximately 10 million deaths or one in six death were reported due to cancer and it has been estimated that about 26 million new cases and 17 million deaths likely to cause by 2030. According to International Agency for Research on Cancer (IARC); breast cancer (BC) is the most commonly diagnosed cancer type, accounting for 1 in 8 cancer diagnosis worldwide.(2) According to GLOBOCAN data 2020, female BC is most commonly diagnosed cancer type with approximately 2.3 million new cases per year.(3) In 2040, the global cancer burden is expected to be 28.4 million cases with a 47% rise from 2020 and it can be further exacerbated by increasing risk factors. Although there is high BC incidence; the drop on mortality is achieved by early diagnosis tools, preventive and curative treatment modalities. For the treatment of BC; radiations, chemotherapy, surgery, immunotherapy and other targeted therapies are being practised. However, the therapies are associated with mild to severe adverse effects. Management of side effects helps in efficacy of treatment and reduces chances of therapy termination due to its severity. Researchers are in search of alternative natural phytochemicals which will be helpful for BC prevention and management but there is need to fulfill the research gap on herbal medicines by substantiating evidences.(4) India being the propagator of Ayurveda; facing challenges on lack of research based evidences for cancer care.(5) For the effective management of BC, there is need to find an integrative potential from Ayurveda and modern science.(6)

Ashwagandha; Withania somnifera (WS) L. Dunal of family 'Solanaceae', commonly known as 'winter cherry' or 'Indian ginseng' is being widely used in Ayurveda therapeutics since more than 5000 years. (Figure 1) Looking to its therapeutic benefits, the annual demand for *Ashwagandha* is estimated as7000 tons. (7) It is a drug having multifaceted pharmacological actions which are clinically proven to be helpful for the management of cancerous conditions and reported to reduce side effects of chemo-radiation therapy.(8) WS being edible and medicinal, a phytochemical derived from such plants are attractive strategy for chemoprevention due to its favorable safety profile.(9)

Withaferin-A (WA); a phytochemical of WS is the first potent anticancer withanolides; later explored

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for its anticancer potential on various cancer cell lines. This review provides evidences on anti-cancer potential of WS for the management of BC.

Material and methods

The review is narrative and presents a critical analysis of in-vitro studies on cancer therapeutics with a focus on medicinal plant *Withania somnifera* (WS) L Dunal and cancer hallmark. It analyses the literature reported on in-vitro activities on breast cancer cell lines. The information was collected from the different online databases namely Web of Science, PubMed, Scopus, Google scholar, CENTRAL, using keywords namely *Withania somnifera* L Dunal; Withaferin A, Ashwagandha, anticancer, in-vitro, breast cancer, cell line etc. with the MeSH terms like 'AND', 'OR', or free text terms. Conference abstracts, editorials, meta-analyses, narrative reviews, and publications written in languages other than English were excluded. Articles of duration 1st January 2011 till 1st January 2021 were included. Limitations found during search were unavailability of some full text articles, information about procurement of *Withania somnifera* sample; its part used for the preparation of extract. Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement has been followed to report the outcomes of this review.

Results

WS contains more than 40 withanolide, 12 alkaloids, and several sitoindosides, withanolides being the most active. (10) It comprises, Withaferin A, Withanolide A and Withanone.

Withaferin A is a most active compound against BC. In vitro studies reports its significant anticancer effect on MCF-7 cells (Table 1), Triple negative breast cancer (TNBC) viz. MDA-MB-231, SUM159, MDA-MB-468, SUM149, SUM159, 231MFP. (Table 2).

Steroid sex hormones play a significant role in onset, progression and prognosis of BC. Estrogen being the malicious culprit in the etiology.(11)As per National Cancer Institute data; the reported evidence for ER +ve BC is 67-80%.(12) It is involved in the evolution and growth of breast tumors through receptor ER. MCF-7 cell line has receptors for the hormones estrogen, progesterone and glucocorticoids. It is commonly used cell lines because it maintains a number of traits resembling mammary epithelium. (13) The reported evidences of WA o ER responsive MCF-7 cell lines are mentioned in table 1.

Figure 2: Prisma Flow chart

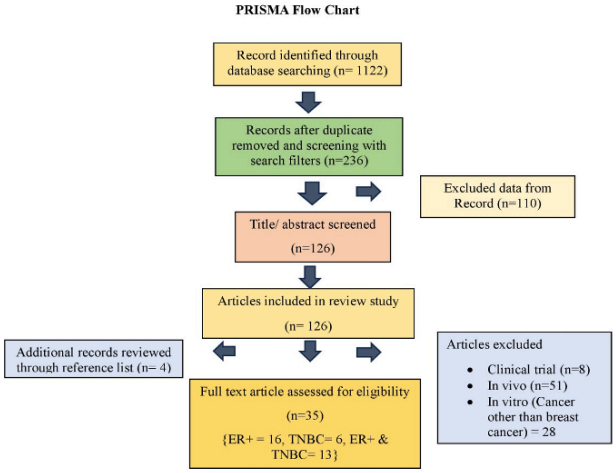

Table 1: Effect of WA on estrogen responsive MCF-7 cells in vitro

SN	Dose of WA (µmol/L)	Mechanism of action	Ref
1	5	Activation of ERK (Extracellular signal-regulated kinase) /RSK-DR5 (Ribosomal S6 Kinase-Death receptor 5	(14)
2	0.5	Inhibition of self-renewal of BCSC	(15)
3	10	Down-regulation of over-expressed ERBB2 and ERBB3 (on standard Lapatinib)	(16)
4	1.25-2.25	S15 phosphorylation of p53 leads to apoptosis	(17)
5	0.5- 2.5	Down-regulation of ERα protein levels	(18)
6	2- 4	Downregulation of tubulin proteins	(19)
7	0.5-10	Inhibition of Pin 1 expression	(20)
8	1-4	NOTCH2 and NOTCH4 activation	(21)
9	2-4	EMT (Epithelial mesenchymal transition) reversal effect	(22)
10	2.5-5	Inhibition of mitochondrial respiration	(23)
11	2.5-5	Over-expression of XIAP protein	(24)
12	1-5	Inhibition of ERK activation, RNA interference of Mcl-1	(25)
13	4	Induction of paraptosis [on standard Amphotericin-B (1.25 µg/ml), penicillin (100 IU/mL) and streptomycin (100 µg/mL]	(26)
14	2-4	Inhibition of mitochondrial fusion	(27)
15	2	Marked increase in levels of cleaved LC3B (LC3B-II) in a time-dependent manner	(28)
16	2.5-5	Inhibition of lysosomal activity and induction of apoptosis	(29)

Progesterone is another ovarian steroid hormone, primarily mediated by progesterone receptor (PR)-A and -B isoforms and is responsible for normal breast development during puberty and in preparation for lactation and breastfeeding. It may act as offender in breast cancer etiology via proliferation of breast cell.

The action of progesterone on BC is controversial as its action is influenced by growth factors, prolactin and estrogen as well. In-vitro studies reports action of WA on PR responsive T47D cell lines by down regulating ER-α protein expression (1.25-2.25 µmol/L)(18) and inhibiting lysosomal activity thus inducing apoptosis (1.25-2.25 µmol/L)(29).

Triple-negative breast cancer (TNBC) cells do not possess estrogen or progesterone, HER2 protein receptors. It is responsible for more than 15–20% of all breast cancers incidences and is of peculiar topic of research interest. It is aggressive in nature, low response to therapeutics and highly invasive nature. MDA-MB-231 cell line are more aggressive, hormone-independent and thus widely used to examine the proliferation and invasion of breast cancer. SUM159 is a mesenchymal cell line used for TNBC experiments. MDA-MB-468 cell line is used to study metastasis, migration and breast cancer proliferation. BT20 cell line is oldest TNBC cell line having hereditary EGFR gene amplification and extremely high EGFR protein expression. SUM149 is an inflammatory breast cancer cell line. 231MFP cells are cultured from MDA-MB-231 cell line which display increased metastasis and tumor growth rates in vivo .

The anti-cancer actions of WA on TNBC are described in table 2.

Table 2: In-vitro action of Withaferin-A on Triple negative breast cancer cell lines

SN	Dose of WA	Mechanism of action	Ref
MDA-MB-231			
1	0.027-2	Inhibit vimentin cytoskeleton, Inhibition of cell invasion Potent anti-invasive action	(30)
2	5	Activation of ERK (Extracellular signal-regulated kinase) /RSK-DR5 (Ribosomal S6 Kinase- Death receptor 5	(14)
3	0.7	Epigenetic effects	(31)
4	0.5, 1	Induction of apoptosis, suppression of proteasomal activities	(32)
5	10	Phosphorylation of residual ERBB2 and activation of MAPK (on standard Lapatinib)	(16)
6	1.25-2.25	G2/M phase cell cycle arrest	(17)
7	2-4	EMT (Epithelial mesenchymal transition) reversal effect	(22)
8	2.5-5	Inhibition of mitochondrial respiration	(23)
9	4	Induction of paraptosis	(26)
10	2	Marked increase in levels of cleaved LC3B	(28)
11	2.5-5	Suppression of XIAP protein	(24)
MDA-MB-231, MDA-MB-468			
12	1-4	NOTCH2 and NOTCH4 activation	(21)
MDA-MB-231, BT20			
13	0.25-2.5	Downregulation of HSF1 and breast cancer susceptibility gene 1	(33)
SUM159			
14	2-4	Downregulation of tubulin proteins	(19)
15	0.5	Inhibition of self-renewal of BCSC	(15)
16	1-5	RNA interference of Mcl-1	(34)
MDA-MB-231, SUM159			
17	2-4	Dysregulation of mitochondrial dynamics	(27)
MDA-MB-231, MDA-MB-468, SUM149, SUM159			
18	2.5-5	Inhibition of lysosomal activity and induction of apoptosis	(29)
231MFP			
19	10	Activation of PP2A activity	(35)

Discussion

BC are primarily of two types i.e., in situ carcinoma and invasive carcinoma. On the basis of hormone receptor status, majorly three main subtypes are found i.e. hormone receptor positive carcinoma ER+ve and PR +ve, human epidermal growth factor receptor positive (HER+), triple-negative breast cancer (lack of expression of ER, PR and HER). Approximately 70% of breast cancers are ER+ve, PR+ve but HER-ve, 15% are HER+ve and 15% are TNBC. TNBC is more aggressive due to non-responsiveness of both endocrinal therapy and chemotherapy.

Withaferin A is considered as the first antitumor withanolides derived from root and leaves of *Ashwagandha*. The chemical structure for WA is WA (4 β,5β,6β,22R)-4,27-dihydroxy-5,6-22,26-diepoxy ergosta-2, 24-diene-1,26-dione) (36). Multiple lines of evidence suggest that withaferin-A can prevent the development of BC of various histotypes like ER+ve, PR+ve, ERPR +ve and TNBC. (Table 1 and 2).

In-vitro anti-cancer mechanisms of Withaferin-A on breast cancer cell lines

- WA increases the phosphorylation of ERK / RSK-DR5 and dysregulation of RSK expression. DR-5 is a death receptor which binds to ligand TRAIL and due to this binding apoptosis occur.14ERK pathway is implicated in diverse cellular physiological processes like growth, proliferation, differentiation, survival etc. Activation of ERK causes cell death by causing changes in cell proliferation and survival.(37)
- WA shows inhibition for self-renewal of BCSC by overcoming NOTCH4 activation and reducing an

expression of *SOX-2* mRNA.(15) NOTCH4 activity is significantly found in BCSC as compared to cancer cells and its over-expression is found in TNBC specimens.(38)Over-expression of *SOX-2 is* found in early stages of breast tumors. It is a transcription factor which activates some genes cascade responsible for cellular proliferation and tumor development.(39)

- One third patients suffering from BC shows the over-expression of receptor tyrosine kinase ERBB2 (ErbB2, HER2) which is common in solid tumors. ERBB3 is an allosteric activator and its elevated levels define progression of many solid tumors. WA down-regulates an over-expressed ERBB2 and ERBB3.(16)
- WA induces S15 phosphorylation; thereby causing activation of p53; a tumor suppressor gene. p53 helps to ceases the cell cycle and provide sufficient time for repair of DNA if it is damaged. After repairs it regulates cell cycle. In severely damaged DNA, it causes cell apoptosis and removes from cell cycle. (17)
- Withaferin A downregulates the ERα protein.ERα is a main type of estrogen receptor which is responsible for regulation of reproduction and physiological activities in the body. The binding of ERα and estrogen activates oncogenic growth pathway in breast cancer.(40)
- In this case, Tubulin is a building block for cell division and movement. Tubulin interacts with DR-5 leading to degradation of cancer cells.
- Tubulin negatively regulates DR5-mediated apoptosis**.** Death receptor-5 (DR5), also known as TRAIL receptor 2 (TRAIL-R2), induces apoptosis in cancer cells. Thus drugs acting by this mechanism are an attractive strategy for cancer therapy. (41)Anticancer drugs like paclitaxel and vinca alkaloids vincristine and vinblastine acts through this mechanism. WA works as anticancer agent by blocking tubulin.
- Pin1 is responsible for cell proliferation. Over-expression of pin-1 is seen in breast cancer and its correlates significantly with the grade of tumor.(42) WA acts as anticancer agent by inhibiting pin-1.
- NOTCH acts as oncogene and a tumor suppressor. It determines cell density and tumerogenecity. NOTCH pathway plays role in over-expression or abnormal genetic expression of the NOTCH receptors and ligands which determine angiogenesis in BC.(43)
- EMT is a complex program which represents one of the “hallmarks of cancer” in which epithelial cells acquire a mesenchymal phenotype and motility through a cascade of biological events.(44) WA exerts EMT reversal effect.
- Mitochondria play an essential role in tumor microenvironment by impaired aerobic respiration which causes changes in cell metabolism leading to expression of oncogene.(45) WA inhibits the mitochondrial respiration.
- XIAP is potent inhibitor of cell death and an attractive onco-therapeutic target due to its ability to suppress caspase activation via both intrinsic and extrinsic pathways. It acts as a signalling intermediate survival of tumor cells. (46)WA suppresses the expression of XIAP in cancer cells thus helps in defeating resistance during chemotherapy and radiation.
- WA down-regulates mitochondrial fusion and fission by suppressing proteins required for the process and increasing mitochondrial volume loss. Mitochondria travel with microtubules and thus disruption of microtubules impairs mitochondrial movement, and causes arrest. WA binds to β-tubulin and disorganizes network of microtubules.
- Lysosomes play a major role in cancer cell death by that including apoptosis, autophagy or necrosis. WA inhibits lysosomal proteolytic activities, blocks autophagic flux and thus exerts antitumor activity in multiple subtypes of breast cancer including luminal A, luminal B, basal, claudin-low, and HER2 subtypes.
- Over expression of Vimentin is seen in epithelial BC and is one of the important prognostic indicators as its mainly found in metastatic cases. WA disturbs the function of vimentin by phosphorylation of ser56 of vimentin.(32)
- WA causes autophagy of cancer cells by mediating cleavage of LC3B.
- WA down-regulates action of HSF1 and breast cancer susceptibility gene 1.HSF1 plays a pleiotropic role in cancer malignancy by causing cell metabolism, proliferation, migration, invasion etc.
- WA triggers apoptosis by RNA interference which causes knockdown of Mcl-1 causing gene silence.
- Cancer cells inactivate PP2A thus reduces its tumor suppressive action. WA maintains healthy cellular function by activating PP2A. (Figure 2)

In Vivo studies reports anti-cancer potential of WA by mechanisms like KLF4 knockdown, inhibition of growth of cells, Inhibition of BCSC in MCF-7 and SUM159.(15) On MDA-MB-231 cell lines, suppression of tumor growth, inhibition of cellular proliferation, increased apoptosis and inhibition of cell motility effect has been reported through animal experimentation. (14,47)Clinical studies also reported effect of Ashwagandha in alleviating chemotherapy-induced toxicity, fatigue and improvement in quality of life in cancer patients.(8)

WS have medicinal as well as nutraceuticals value.(48) It possess analgesic,(49) anti-inflammatory, (50) antioxidant,(51)antibacterial,(52) anticonvulsant, (53) neuro-protective,(54) immunomodulatory action, (55) anti-ageing,(56) sedative(57) actions. Thus, helpful to make body ready for chemo-radiation and combat against side effects of therapies.

During postmenopausal phase, due to hypo-functioning of ovaries; estrogen deficiency is predominantly seen causing osteoporotic changes which exacerbated by calcium deficiency. Estrogen Replacement Therapy (ERT) is effective against these changes. *Ashwagandha* contains estrogen like withanolides, particularly WA which significantly prevents bone loss. Moreover, it reduces the urinary excretion of calcium and phosphorous and thus maintains the serum calcium levels. (58)

In Ayurvedic treaties, the BC can be correlated with *Stan-arbuda*. According to Ayurveda science, breast tissue is predominantly composed of *mamsa* and *meda dhatu*. The breast milk is said to be by-product of *Rasa-dhatu*. The *Rasa* is vitiated by *guru* (heavy), stale, excessive oily, frequent eating, taking large quantity of food quantum, over-thinking, stress etc.(59) In contemporary lifestyle, unhealthy diet, junk food, lack of exercise, stressful life, intake of hormone pills, radiation, alcohol consumption, etc contributes to this causative factors.

When body is vitiated by *tridosha*, (*vata, pitta, kapha*); it causes *sanga* (obstruction) of milk ducts, which leads to accumulation of *tridosha* forming a mass like structure; termed as *stana-arbuda*. *Ashwagandha* possess *tikta-kashya* (bitter- astringent) in *rasa* thus helpful for removing accumulated *ama dosha* from *rasavaha strotasa*. Being *ushna* in *veerya* it pacify aggravated *vata-kapha dohsa*. *Aswagandha* is *atishukrala*, i.e. it corrects the functioning of *shukra* by regulation of hormones. It is *bruhana* (nourishing) it helps in proper formation of *rasa dhatu* which further nourishes *rakta-mamsa-meda-asthi-majja* and *shukra dhatu*. Being *balya* (tonic) thus helps to provide strength to tissue specially *mamsa* and *shukra*. *Ashwagandha* possess *rasayana* (adapto-immuno-neuroendocrine regulator) effect thus helps in rejuvenation of cells.(60) *Ashwagandha* can be given with milk, warm water, ghee, honey, etc. according to the symptoms and its predominant *dosha*. Many preparations are available in the market such as *Ashwagandha rasayana*, *Ashwagandha ghrita*, *Ashwagandha avaleha*, *Ashwagandharishta*, etc. It should prescribe with proper examination of *prakruti* (body constitution), *agni* (digestive fire), *dosha* (patho-physiological entities), *desha* (type of area and environmental factors) and *vaya* (age of patient), etc.

The vegetative phase of plant possesses higher WA as compared to reproductive phase or at maturity. During vegetative phase, WA content of leaves and roots are 1.099% and 0.1242% respectively. Geographical variation does not cause any significant effect on WA content.(61) Collection of roots for therapeutic purpose is advised on full moon days and in summer seasons (*greehsma*).(62)

Ashwagandha has no serious reported adverse events. Solanaceae family drugs if taken without appropriate indications and excessive dose; possess mild to moderate adverse event such as nausea, epigastric pain, giddiness, drowsiness, decreased appetite, hallucinogen, cough, cold, gastritis, flatulence, loose stools, constipation, etc.(63)

Conclusion

Withaferin A, a phytochemical derived from *Withania somnifera* is helpful for chemo-prevention by imparting mechanisms like apoptosis, activation of ERK and RSK pathways, S15 phosphorylation of p53, down regulation of ERα and tubulin protein, activation of NOTCH2 and NOTCH4, inhibition of mitochondrial respiration, inhibition of lysosomal activity, etc. It also sensitizes resistant cancer cells to existing chemotherapeutic agents. *Ashwagandha* is a natural medicinal resources, easily available, cost effective and easy to administer in various dosage forms. The reported preclinical anti-cancer potential against breast cancer; needs further exploration through integrative oncology settings for its clinical efficacy.

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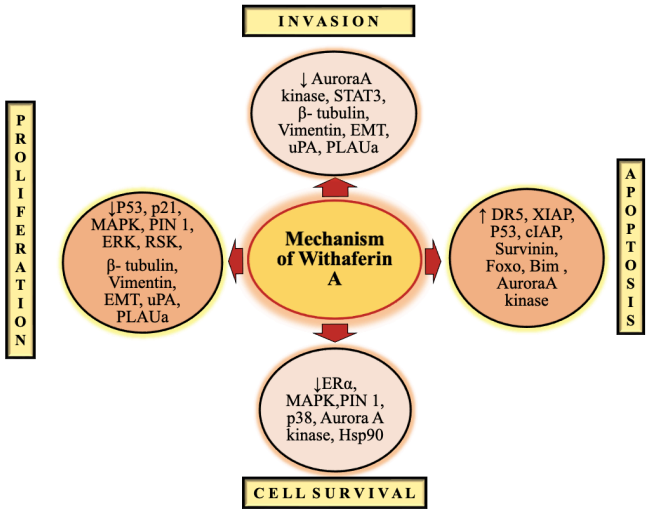
List of Abbreviations

Abbreviations	Definition
BC	Breast cancer
WS	Withania somnifera
WA	Withaferin-A
ER +ve	Estrogen receptor +ve
PR +ve	Progesterone receptor +ve
HER2	Human epidermal growth factor receptor 2
TNBC	Triple-negative breast cancer
ERT	Estrogen replacement therapy
ERK	Extracellular signal receptor kinase
RSK	Ribosomal S6 Kinase
TRAIL	TNF- related apoptosis-inducing ligand
DR 5	Death receptor 5
BCSC	Breast cancer stem cell
EMT	Epithelial mesenchymel transition
XIAP	X-linked inhibitor of apoptosis protein
KLF4	Kruppel-like factor 4
ERα protein	Estrogen receptor alpha protein
LC3	Microtubule-associated protein 1A/1B-light chain 3
HSF1	Heat shock transcription factor 1
Mcl-1	Myeloid leukemia 1
PP2A	Protein phosphatase 2A
ERBB2	Erb-b2 receptor tyrosine kinase
ERBB3	Erb-b2 receptor tyrosine kinase 3
PIN1	Peptidyl-prolyl isomerase

Figure 1: Ashwagandha (Withania somnifera L Dunal)

Figure 3: Anti-cancer mechanism of Withaferin A against breast cancer



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