

Andrographolide a Potential Therapeutic Drug against Breast Cancer: A Review

Sheethal S. Kumar^{1*}, John E. Thoppil²

¹Research Scholar, Cell and Molecular Biology Division, Department of Botany, University of Calicut, Kerala, India

²Professor, Cell and Molecular Biology Division, Department of Botany, University of Calicut, Kerala, India

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ABSTRACT

Breast cancer is the leading cause of cancer death among females. It occurs due to the uncontrolled proliferation of breast epithelial cells. Plant-derived drugs are more efficacious than synthetic drugs since it lacks side effects. Andrographolide is an active principle of the herb *Andrographis paniculata* (Burm. f.) Nees. It is a potent therapeutic drug against breast carcinoma. It induces breast cancer cell-cycle arrest at the G0/G1 phase via the induction of cell-cycle inhibitory protein p27 and decreases the expression of cyclin-dependent kinase 4 (CDK4). It also activates the intrinsic apoptotic pathway, via the induction of Bax and Bak. It enhances caspase production and p53 gene expression. Andrographolide inhibits angiogenesis and metastasis of breast carcinoma. Andrographolide nanoparticle production and synergetic therapies of andrographolide, along with other chemotherapeutic drugs, is a breakthrough in breast cancer treatment.

Keywords: Andrographolide, Apoptosis, Breast carcinoma, Cell cycle arrest, SARS-CoV-2.

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INTRODUCTION

India enjoys the privilege of having time tested traditional systems of medicines based on natural products. Plant-based products have been in use for medicinal, therapeutic, or for other purposes right from the dawn of history.¹ The family Acanthaceae is commonly known as “Acanthus” family, and includes several medicinal plants. The genus *Andrographis* Wall. ex. Nees is a leading member of them. It is a tropical Asian genus represented by 28 taxa in India, of which 23 are endemic. The members of the genus are mainly concentrated in southern states of India, namely, Tamil Nadu, Andhra Pradesh, and Karnataka.² Species of *Andrographis* are used in the Indian system of medicines such as *Ayurveda*, Homeopathy, Naturopathy, *Amchi*, *Siddha*, and *Unani*.³ *Andrographis paniculata* (Burm.f.) Nees. is the type species of the genus *A.*, and it is a well known medicinal plant. It is known as the ‘king of bitter’ because of the extremely bitter taste in all parts of the body. As an *Ayurvedic* plant, it is known as “Kalmegh” or “Kalmegha” meaning “dark cloud.”⁴ Aerial parts of the plant are traditionally used to treat cancer.³

The active principle of *A. paniculata* is andrographolide, a diterpenoid lactone. It is a potential therapeutic agent against human cancer. It has reported anticancer and immunostimulatory properties.⁴ The compound exerts direct anticancer activity on cancer cells via cell-cycle arrest at the

G0/G1 phase through induction of cell-cycle inhibitory protein p27 and decreased expression of CDK4.⁵ The present paper is a review of the therapeutic potential of andrographolide against breast cancer. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females.⁶ Andrographolide has the hypolipidaemic effect, and recent studies revealed that this activity of andrographolide could be utilized to prevent the resistance in the treatment of ER-positive breast cancer.⁷ Cell cycle arrest, apoptosis, anti-angiogenesis, synergetic therapeutic potential, etc., of andrographolide and some of its derivatives, are discussed in this scientific exploration.

Andrographolide induces G1/G0 Cell Cycle Arrest

Cyclin and cyclin-dependent kinases (CDK) are the two subunits of the protein series involved in cell cycle progression, in which cyclin is the regulatory component, and CDK is the catalytic subunit. CDKs are the regulators of cell cycle checkpoints. Among which CDK4 and CDK6 are the targets of anticancer drugs since they regulate the cell cycle progression at the G1 restriction point.⁸ The p27 and p21 are proteins that have a negative effect on cell proliferation. It inhibits the activities of cyclin E/CDK2 and cyclin A/CDK2, and they seem to activate cyclin D/CDK complexes. In proliferating cells, p27 is prevalently bound to cyclin D/CDKs, whereas, in G1-arrested cells, p27 is found in complexes with cyclin E/CDK2.⁹

*Author for Correspondence: sheethalskumar121@gmail.com

Breast cancer develops due to the uncontrolled proliferation of breast epithelial cells. Loss of function of the p27 gene is one of the reasons for breast cancer. The p27 gene is located in human chromosome 12p 13th locus. Biallelic mutation of the p27 gene is rare in the case of breast carcinoma.¹⁰ The p27 protein levels are high in quiescent cells and undetectable or very low in proliferative cells. The p27 mRNA seems to be constant throughout the cell cycle, but the p27 protein level is high in the G1 phase and decreases during the end of the G1 phase.⁹ During the end of the G1 phase, p27 protein is degraded via the ubiquitin-proteasome pathway. The human ubiquitin-conjugating enzymes are Ubc2, and Ubc3, specifically involved in the ubiquitination of p27.¹¹ Breast cancer patients with low expression of p27 have a high mortality rate than patients with high expression of p27.¹² Induction of p27 in breast carcinoma is an effective strategy for breast cancer treatment.

Andrographolide blocks breast cancer cell proliferation via the induction of p27 and concomitant decrement in the levels of CDK4. Fluorescence-activated cell sorting (FACS) analysis and western blot analysis were conducted in MCF-7 breast cancer cell lines after treating with andrographolide. FACS analysis showed that cells were arrested at the G0/G1 phase. Western blot analysis confirmed the observation of FACS analysis by detecting the presence of cell cycle inhibitor proteins p27. Induced p27 proteins bind with CDK4/ D1 complex and prevent the phosphorylation of retinoblastoma. This prevents the release of elongation factor and transcription of S phase proteins.¹³

Andrographolide showed a time- and concentration-dependent inhibitory effect on MDA-MB-231 breast cancer cell proliferation. The treatment did not affect normal breast epithelial cells, MCF10A (> 80%). The number of cells in S, as well as the G2/M phase, was increased after 36 hours of treatment.¹⁴ MDA-MB-231 cells exposed with andrographolide for 24 hours showed significant cell cycle arrest at G2/M phase.¹⁵ Andrographolide also inhibits breast cancer growth via the inhibition of hypoxia-inducible factor-1 through the phosphatidylinositol 3-kinase/AKT pathway.¹⁶

Induction of Apoptosis

Apoptosis is an active process of programmed cellular suicide. This process involves an epigenetic reprogramming of the cell that results in an energy-dependent cascade of biochemical and morphologic changes within the cell. This process results in the death and elimination of cells.¹⁷ There are multiple pathways for apoptosis. The two major pathways are intrinsic pathway mediated by mitochondria and extrinsic pathway via death receptor (Fas receptor). Both the pathways converge finally to the activation of caspases.¹⁸ The caspases constitute a family of cysteine proteases, peptidases that have a cysteine residue at its catalytic site, and cleave the target protein sites next to aspartic acid residues.¹⁹ Cytotoxic drugs usually work by the activation of the intrinsic apoptotic signaling pathway.¹⁸ The Bcl-2 family proteins are key regulators of the intrinsic pathway, which include both anti- and pro-apoptotic proteins.²⁰ Anti-apoptotic proteins are Bcl-2 and Bcl-xL, whereas Bax and Bak are pro-apoptotic proteins.²¹

Andrographolide is an excellent cytotoxic drug against breast carcinoma. It induces apoptosis in the human breast cancer cell line by increasing the expression of p53, Bax, caspase-3, and decreases the expression of Bcl-2.²² Andrographolide induces cellular apoptosis via changes in cellular morphology and chromatin condensation, a hallmark of apoptosis. It can restrain proliferation and stimulate nuclear DNA fragmentation resulting in augmentation of apoptosis in MDA-MB-231 cells. Additionally, it may cause mitochondrial membrane damage and also elevate the production of ROS. Increased production of ROS decreases mitochondrial membrane potential (MMP). ROS production and loss of MMP increases oxidative stress, which can induce apoptosis in breast cancer cells.¹⁵ Harjotaruno *et al.*²² showed that andrographolide induced apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increasing expression of p53 Bax, caspase-3, and decreasing the expression of Bcl-2. Beesetti *et al.*²³ treated andrographolide against A431, MDA-MB231, breast cancer cell lines, and SKOV-3 ovarian cancer cell lines. Andrographolide treatment inhibited NFκB activation, which induced caspase-8-mediated apoptosis.

Inhibit Metastasis

Metastasis is a multistage process in which tumor cells escape from the primary sites and survive in circulation. These cells seed at a distant place and develop a new tumor. The tumor microenvironment has a significant role in modulating the metastatic ability of most cancers.²⁴ Matrix metalloproteinases (MMPs) play a pivotal role in metastasis and tumor invasion. They are zinc-dependent extracellular matrix (ECM) remodelling endopeptidases.²⁵ Nuclear factor-κB signaling is responsible for the expression of matrix metalloproteinase-9 expression.²⁶ Several reports reveal that andrographolide inhibits the NF-κB signaling.²⁷⁻²⁹ Zhai *et al.*³⁰ investigated that andrographolide prevents metastasis of MDA-MB-231 breast cancer cells via inhibiting factor-κB-dependent matrix metalloproteinase-9 expression. Beesetti *et al.*²³ also reported andrographolide-mediated NF-κB pathway inhibition in MDA-MB231, breast cancer cell lines, and SKOV-3 ovarian cancer cell lines.

Claudin-1 is an integral membrane protein residing at the tight junction.³¹ Dhawan *et al.*³² reported that claudin-1 expression increased with colon cancer proliferation and metastasis. Andrographolide suppresses claudin-1 expression via the p38 signaling pathway.³³ Some cancer cells are characterized by increased intercellular pH and lead to cancer cell migration as well as invasion. The process of pH homeostasis plays an important role in controlling cell proliferation. Andrographolide maintains the intercellular pH by regulating the membrane transporters and thereby prevents cancer metastasis.³⁴

Anti-Angiogenesis

Angiogenesis is the process of generating new capillary blood vessels and leads to neovascularization.³⁵ Vascular endothelial growth factor (VEGF), also known as vascular permeability

factor (VPF) is the key protein responsible for angiogenesis. Anti-VEGF strategies to treat cancers were designed to target the pro-angiogenic function of VEGF and thereby inhibit neovascularization.³⁶ VEGF expression in breast cancer is well documented, and it is produced by both macrophages, and cancer cells in breast carcinoma.³⁷ VEGF and its receptors are co-expressed on breast cancer cells, suggesting that both paracrine and autocrine VEGF pathways play a role in breast cancer progression.³⁸ Anti-VEGF drugs target angiogenesis and breast carcinoma. VEGFR2 is the receptor for VEGFA. VEGFA-VEGFR2 interaction causes receptor activation leading to the activation of tyrosine kinase, which in turn, switch-on downstream signaling molecules that mediate endothelial cell migration, proliferation, survival, and vascular permeability.³⁹ Molecular-docking module and biochemical analysis conducted by Kajal *et al.*⁴⁰ identified andrographolide as one of the best docking molecules that bind to ATP-binding pocket of VEGFR2 and inhibits its kinase activity, thereby inhibiting tumor angiogenesis. Andrographolide reduces VEGF and its receptor expression. VEGF mRNA level in BI6F-10 cell line showed a reduced level of expression in the presence of andrographolide.⁴¹

Andrographolide Derivatives against Breast Carcinoma

The chemical name of andrographolide is 3 α , 14, 15, 18-tetrahydroxy-5 β , 9 β H, 10 α -labda-8, 12-dien-16-oic acid γ -lactone, and its molecular formula is C₂₀H₃₀O₅.⁴² The structure of andrographolide comprises an α -alkylidene γ -butyrolactone moiety, two olefin bonds [Δ 8 (17) and Δ 12 (13)], three hydroxyls at C-3, C-19, and C-14 and highly substituted trans decalin. Of the three hydroxyl groups, the one at C-14 is allylic in nature, and the others at C-3 and C-19 are secondary and primary, respectively.⁴³

Derivatives of andrographolide showed good activity against many diseases, especially cancer.⁴⁴ Menon and Bhat⁴⁵ proved the anticancer potential of semi-synthetic andrographolide against A549 (ATCC) (NSCL cancer) cell lines. Liu *et al.*⁴⁶ synthesized the thioether derivatives of andrographolide and proved their inhibitory effect against cancer cells. Poerwono *et al.*⁴⁷ modified andrographolide to improve its anticancer potencies. After the protection of the two hydroxyl groups present at C-3 and C-19 to give 3, 19-isopropylidene and 3,19-benzylidene andrographolides, the remaining hydroxyl group at C-14 of andrographolide was treated with acid anhydride or acid chloride under base condition. The reactions gave only 14-dehydro-andrographolide, as well as unidentified diacyl compounds in place of the target molecule 14-O-acyl andrographolide. An alternative procedure using neat acetic anhydride under reflux gave the acetyl derivatives. The resulted compounds exhibited cytotoxic activity against MCF-7 breast cancer cells with better growth inhibition than the parent compound andrographolide.

Benzylidene derivatives of andrographolide such as 3,19-(2-bromobenzylidene) andrographolide and 3,19-(3-chloro-4-fluorobenzylidene) andrographolide induced a consistent G₁ phase arrest in MCF-7 cells, down-regulated

CDK4 level in MCF-7 cells upon treatment with 1 and 7 μ M for 72 hours but did not alter CDK1 level.⁴⁸ Jada *et al.*⁴⁹ synthesized isopropylideneandrographolide, 14-acetyl-3,19-isopropylideneandrographolide, and 14-acetylandrographolide, the semisynthetic derivatives of andrographolide. Among which isopropylideneandrographolide and 14-acetylandrographolide showed a non-specific phase of the cell cycle arrest in MCF-7 cells treated at different intervals with different concentrations.

Chakraborty *et al.*⁵⁰ synthesized dispiroandrographolide derivatives from isatin/acenaphthoquinone, N-benzylglycine, and andrographolide via azomethine ylide cycloaddition reaction. The cytotoxic effect of the synthesized molecules has been studied against the MCF-7 breast cancer cell line. The compounds induced apoptotic cell death decreased polarization of cell mitochondria and increased reactive oxygen species production. Using FACS and western blot analysis, the compounds were observed to block the cell cycle at S phase. DRF3188 is a novel semi-synthetic derivative synthesized by Sathyanarayana *et al.*¹⁴ and had reported its anticancer potential against MCF-7 breast cancer cell lines.

Neoandrographolide is a natural analog of andrographolide isolated from *A. paniculata*.⁴³ It is the second-largest diterpenoid group widely distributed in the plant.⁵¹ Ethanol extract of *A. paniculata* plant contains 2.13 ppm (4.43 μ M) neoandrographolide and shows an inhibitory effect in MCF-7 cells with higher doses.⁵²

Andrographolide Nanoparticles against Breast Carcinoma

Nanoparticles represent an effective drug delivery system. It delivers several classes of drugs such as anticancer agents, antihypertensive agents, immunomodulators, and hormones. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles are extensively utilized for the synthesis of nanoparticles since they are biodegradable.⁵³ PLGA nanoparticulation of andrographolide increased its anticancerous activity threefold. Chitosan coated nanoparticles of andrographolide induced cell cycle arrest, increased cellular toxicity, and activated apoptotic pathways in MCF-7 breast cancer cell lines.⁵⁴

Parveen *et al.*⁵⁵ synthesized solid lipid nanoparticles (SLN) of andrographolide. SLNs of andrographolide showed more anti-tumor activity than normal andrographolide when treated against MCF-7 breast carcinoma. SLNs, when attached to cell membranes, altered the homeostasis of cells and which further added the cytotoxicity of treated cells.

Synergetic Therapies of Andrographolide

Andrographolide, along with other chemotherapeutic drugs, is a breakthrough in breast cancer treatment. Cisplatin and carboplatin are platinum complexes that have broad-spectrum activity against epithelial cancers.⁵⁶ It demonstrates significant activity against cancers of the lung, head, neck, esophagus, bladder, cervix, and endometrium.⁵⁷ Andrographolide sensitizes breast cancer cells to cisplatin and exerts synergistic effects in the treatment of cancer.⁵⁸ Ananthapur *et al.*⁵⁹ investigated the synergetic effect of andrographolide along

with carboplatin against triple-negative MDA-MB-231 breast cancer cell lines. They found that andrographolide reduced the proliferation of MDA-MB-231 cells and also showed enhanced anti-proliferative effects when treated in combination with carboplatin. Andrographolide was also found to enhance the levels of DNA damage repair proteins during co-treatment of cells with andrographolide and carboplatin.

Pheophorbide is the active ingredient of rat taro extract (*Typhonium flagelliforme*). It inhibits cell proliferation via DNA fragmentation and cell apoptosis induction.⁶⁰ It inhibits breast tumor proliferation via caspase-dependent and independent pathways.⁶¹ A combination of pheophorbide and andrographolide showed anticancer effectiveness in MCF-7 breast cancer cell lines at 4 μ M pheophorbide doses and 24 μ M andrographolide.⁶⁰ Kang *et al.*⁶² co-delivered andrographolide and doxorubicin via liposome to an orthotopic breast tumor mouse model. The synergistic effect of doxorubicin and andrographolide inhibits breast tumor growth and prevents lung metastasis.

Radiotherapy (RT) is a curative treatment option for some malignant tumors, but it is a failure in the case of metastasis.⁶³ Andrographolide produces a radiation-induced cytotoxic effect in Ras-transformed cell *in vitro* and *in vivo* via attenuating the activity of NF- κ B.⁶⁴ The combined action of andrographolide and radiation significantly inhibited metastasis in Ras-transformed cells via suppression of ERK-mediated MMP-2 (matrix metalloprotease) activity.⁶³

Andrographolide to protect Cancer Patients from SARS-CoV-2 Infections

Recent studies had revealed the discovery of potential drugs by computational methods, which act as the therapeutic targets for SARS-CoV-2 infection. The natural compound, 14-deoxy-11,12-didehydroandrographolide from *A. paniculata* was found to be acting as a potential antiviral, anti-inflammation, and anti-tumor compound. The andrographolide derivative exhibited a relatively high binding affinity to the three target proteins, Nsp1, Nsp3c, and ORF7. Recent studies had also revealed neoandrographolide to be a potential TMPRSS2 inhibitor and, thus, predicted it also to act as an anti-virus natural compound. Herbal medicines containing andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide from *A. paniculata* as major components might be meaningful for the treatment of SARS-CoV-2 infections.⁶⁵

CONCLUSION

The medicinal herb, *Andrographis*, is frequently used in South Asian countries, including India, for preventing and treating the common cold and flu (influenza). The herbal formulations of the *Andrographis* complex are used traditionally to help maintain a healthy immune system function and support a healthy respiratory system function. Andrographolide is the vital principle of *A. paniculata* and plays a significant role in cancer prevention, especially breast cancer. It inhibits breast cancer cell proliferation by arresting the cell at the G1/G0 phase

via up-regulating the expression of p27 and downregulating the expression of CDK4. It induces apoptosis in these arrested cells via activating the intrinsic apoptotic pathway. Metastasis and angiogenesis are the two dangerous situations in breast cancer and the leading cause of death. Andrographolide inhibits both these dangerous conditions of breast cancer. Recent studies revealed that modified andrographolides and its nanoparticles are more efficient in preventing cell proliferation than the parent compound andrographolide. Andrographolide enhanced the curative potentials of chemotherapeutic drugs in synergetic therapies. Thus, it can be concluded that andrographolide will be a potential breakthrough in breast cancer treatment. It can even be used effectively to check SARS-CoV-2 infections in cancer patients.

Compliance with Ethical Standards

- Disclosure of potential conflicts of interest: Author A-Ms. Sheethal S. Kumar declares that she has no conflict of interest. Author B-Prof. John E. Thoppil declares that he has no conflict of interest.
- Research involving human participants and/or animals: This article does not contain any studies with human participants or animals performed by any of the authors.
- Informed consent: Informed consent was obtained from all individual participants included in the study.

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