A Novel Hybrid Approach To Wireless Channel Estimation Using Vision Transformers (ViTs)

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Abstract

Background: Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide, and the toxicity and drug resistance associated with conventional chemotherapy have renewed interest Ain plant-derived bioactive combinations. Withania somnifera (Ashwagandha; A) and Camellia sinensis (Green tea; G) are two widely studied medicinal plants individually reported to possess anticancer, pro-apoptotic and antioxidant-modulating properties.
Objective: The present study was designed to evaluate the in vitro cytotoxic, apoptotic, pro-oxidant and anti-migratory potential of a hydroalcoholic combination extract of Ashwagandha and Green tea (designated A.G.) against the human breast adenocarcinoma cell line MCF-7.
Methods: MCF-7 cells were treated with increasing concentrations of A.G. (10-400 µM) and cell viability was assessed by the MTT assay. Nuclear and membrane morphological changes associated with apoptosis were examined using acridine orange/propidium iodide (AO/PI) dual fluorescent staining. Intracellular reactive oxygen species (ROS) generation was measured using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe. Cell migratory capacity was evaluated using the in vitro wound-healing (scratch) assay at 0, 12 and 24 h.
Results: A.G. produced a concentration-dependent decrease in MCF-7 cell viability (IC50 ≈ 100 µM at 24 h), with a non-significant effect at 10 µM and highly significant reduction in viability from 25 µM upward ($p<0.05$ to $p<0.001$). AO/PI staining of cells treated with A.G. (100 µM) revealed a marked shift from uniform green (viable) fluorescence in control cells to intense orange-red (PI-positive) fluorescence, indicating apoptotic/late-apoptotic and membrane-compromised populations. A.G. treatment produced a significant increase in intracellular ROS level relative to untreated control ($p<0.001$), consistent with a pro-oxidant mechanism of cell killing. In the wound-healing assay, A.G.-treated MCF-7 cells showed visibly delayed wound closure at 12 and 24 h compared with control, indicating suppression of cell migration.
Conclusion: The hydroalcoholic Ashwagandha-Green tea combination extract (A.G.) exerts concentration-dependent cytotoxic and pro-apoptotic activity against MCF-7 breast cancer cells, mediated at least in part through excessive intracellular ROS generation, and also inhibits their

migratory potential. These findings support further mechanistic and in vivo evaluation of A.G. as a candidate adjunct phytotherapeutic agent in breast cancer.

Keywords:BWithania somnifera; Camellia sinensis; Ashwagandha; Green tea; MCF-7; MTT assay; apoptosis; reactive oxygen species; cell migration; breast cancer

1. Introduction

Breast cancer is the most frequently diagnosed malignancy and a leading cause of cancer death among women globally. Despite advances in surgery, radiotherapy, hormonal therapy and chemotherapy, the clinical benefit of conventional cytotoxic agents is often limited by systemic toxicity, poor tumor selectivity and the emergence of drug resistance, which has intensified the search for safer, plant-derived anticancer agents that can be used alone or as adjuncts to standard therapy [1].

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, is a cornerstone herb of Ayurvedic medicine whose roots and leaves are rich in steroidal lactones (withanolides), including withaferin A and withanone. Ashwagandha extracts and isolated withanolides have been reported to inhibit proliferation and induce programmed cell death in several breast cancer cell lines, including MCF-7 and MDA-MB-231, largely through generation of reactive oxygen species (ROS), modulation of the Bcl-2/Bax axis, and activation of intrinsic mitochondrial apoptotic pathways [2-6].

Camellia sinensis (green tea) is one of the most widely consumed beverages worldwide and is a rich source of catechins, particularly (-)-epigallocatechin-3-gallate (EGCG), the most abundant and biologically active polyphenol in green tea. Green tea catechins and crude extracts have been shown to suppress proliferation, promote apoptosis, and inhibit the migratory and invasive behaviour of breast cancer cells, including MCF-7, through p53-dependent cytotoxicity, downregulation of VASP and Rac1 activity, and modulation of EGFR/CD44 signalling [7-11].

Given that Ashwagandha and green tea individually exhibit complementary pro-apoptotic, pro-oxidant and anti-migratory activities, and that combinations of these two botanicals have already been explored for enhanced antioxidant potential [12], we hypothesised that a combined hydroalcoholic extract of Ashwagandha and green tea (hereafter referred to as A.G.) may produce a more robust anti-breast-cancer effect than either agent alone. In the present study, we evaluated the effect of A.G. on (i) cell viability by the MTT assay, (ii) apoptotic morphology by AO/PI dual staining, (iii) intracellular ROS generation, and (iv) cell migration by the wound-healing assay, in the estrogen-receptor-positive human breast cancer cell line MCF-7.

2. Materials And Methods

2.1 Plant material and preparation of hydroalcoholic combination extract (A.G.)

Authenticated roots of Withania somnifera (Ashwagandha, A) and dried leaves of Camellia sinensis (Green tea, G) were procured and shade-dried, and the dried material was pulverized to a coarse powder. The powdered plant material was subjected to extraction with a hydroalcoholic solvent system (ethanol:water) using a Soxhlet/maceration procedure. The two extracts were combined in a fixed ratio to obtain the composite extract designated A.G., which was concentrated under reduced pressure, lyophilized, and stored at 4°C until use. A stock solution of A.G. was prepared in dimethyl sulfoxide (DMSO; final DMSO concentration ≤0.1% v/v in culture medium) and diluted in complete culture medium to obtain the working concentrations used in this study (10, 25, 50, 100, 200 and 400 µM).

2.2 Cell line and culture conditions

The human breast adenocarcinoma cell line MCF-7 was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained at 37°C in a humidified atmosphere of 5% CO2. Cells in the logarithmic growth phase were used for all experiments.

2.3 MTT cell viability assay

Cell viability was assessed by the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) colorimetric assay as originally described by Mosmann [13]. Briefly, MCF-7 cells were seeded in 96-well plates (5×10^3 cells/well) and allowed to adhere for 24 h, followed by treatment with A.G. at 10, 25, 50, 100, 200 and 400 µM, or vehicle control, for 24 h. MTT reagent (0.5 mg/mL) was then added to each well and incubated for 3-4 h at 37°C to allow formation of formazan crystals, which were solubilised in DMSO, and absorbance was recorded at 570 nm using a microplate reader. Percentage cell viability was calculated relative to the untreated control (set at 100%), and the half-maximal inhibitory concentration (IC50) was derived from the dose-response curve.

2.4 AO/PI dual staining for apoptosis

Morphological features of apoptosis were assessed by acridine orange/propidium iodide (AO/PI) dual fluorescent staining [14,15]. MCF-7 cells were seeded in chamber slides/plates and treated with A.G. (100 µM) or vehicle control for 24 h. Cells were then stained with an AO/PI dye mixture and immediately observed under a fluorescence microscope at 10X objective magnification. Viable cells display uniform green (AO-positive) nuclear fluorescence with intact membranes, whereas apoptotic/necrotic cells with compromised membrane integrity take up PI and show orange-red fluorescence.

2.5 Measurement of intracellular reactive oxygen species (ROS)

Intracellular ROS generation was measured using the cell-permeable fluorogenic probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), which is deacetylated by intracellular esterases and oxidized by ROS to the highly fluorescent 2',7'-dichlorofluorescein (DCF), as previously applied to green tea polyphenol-treated MCF-7 cells [16]. MCF-7 cells were treated with A.G. (100 µM) or vehicle control for 24 h, incubated with DCFH-DA, and fluorescence was captured under a fluorescence microscope at 20X objective magnification. Relative fluorescence intensity (proportional to intracellular ROS level) was quantified using image analysis software and expressed relative to control.

2.6 Wound-healing (scratch) migration assay

The effect of A.G. on MCF-7 cell migration was evaluated using the in vitro wound-healing/scratch assay [8,9]. Confluent monolayers of MCF-7 cells were scratched with a sterile micropipette tip to create a uniform wound, washed with PBS to remove detached cells, and then incubated in the presence or absence of A.G. (100 µM) in serum-reduced medium. Wound closure was monitored and photographed at 0, 12 and 24 h using a phase-contrast inverted microscope, and the extent of wound closure was compared qualitatively and quantitatively between control and A.G.-treated groups.

2.7 Statistical analysis

All experiments were performed in at least triplicate, and data are expressed as mean ± standard deviation (SD)/standard error of mean (SEM). Statistical comparisons between control and treatment groups were performed using one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test, or Student's t-test for two-group comparisons, using GraphPad Prism software. A p-value <0.05 was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$; ns = not significant).

3. Results

3.1 A.G. reduces MCF-7 cell viability in a concentration-dependent manner (MTT assay)

Treatment of MCF-7 cells with A.G. for 24 h produced a clear concentration-dependent reduction in cell viability (Figure 1). Compared with the untreated control (100% viability), 10 µM A.G. produced only a modest, statistically non-significant reduction in viability (~91%, ns). From 25 µM onward, viability declined significantly and progressively: ~79% at 25 µM ($p<0.05$), ~68% at 50 µM ($p<0.01$), ~51% at 100 µM ($p<0.001$), ~38% at 200 µM ($p<0.001$) and ~35% at 400 µM ($p<0.001$). Based on this dose-response profile, an IC50 of approximately 100 µM was identified and used as the working concentration for the subsequent mechanistic assays.

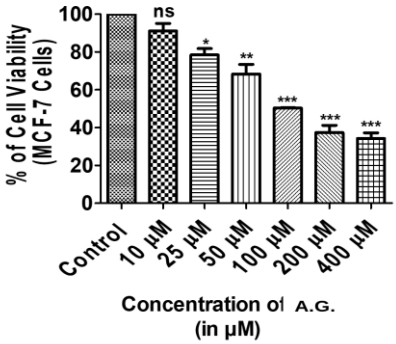

Figure 1. Effect of A.G. on the viability of MCF-7 cells assessed by MTT assay after 24 h treatment across a concentration range of 10-400 µM. Data are expressed as % of cell viability relative to untreated control. ns = not significant, *p<0.05, **p<0.01, ***p<0.001 vs. control.

3.2 A.G. induces apoptotic morphological changes in MCF-7 cells (AO/PI assay)

AO/PI dual staining was used to visualize apoptotic/necrotic morphology in control and A.G. (100 µM)-treated MCF-7 cells (Figure 2). Control cells displayed predominantly uniform green (AO-positive) fluorescence with intact, well-organized nuclei and minimal PI uptake, indicative of high cell viability. In contrast, A.G.-treated cells showed a markedly reduced AO (green) signal accompanied by a pronounced increase in orange-red PI fluorescence in both the PI and merged (AO/PI) channels, reflecting loss of membrane integrity and nuclear changes consistent with apoptosis/late apoptosis-necrosis in a substantial proportion of the treated cell population.

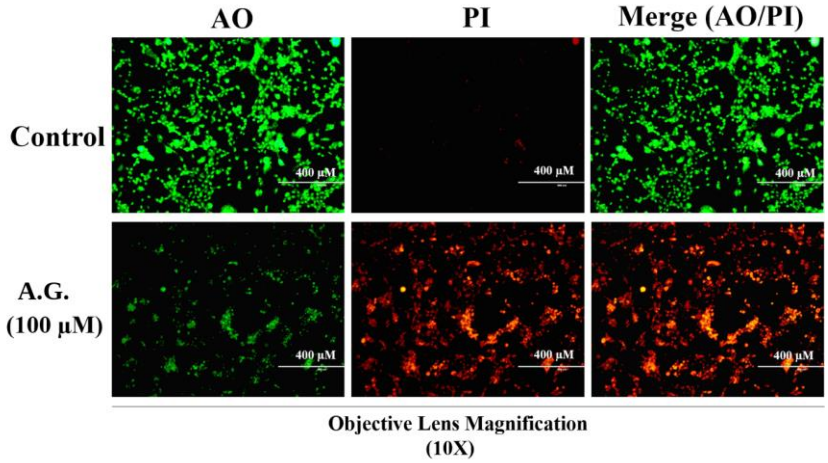

Figure 2. Representative fluorescence images of AO/PI dual staining of MCF-7 cells: control (untreated) versus A.G. (100 µM)-treated cells (24 h), showing AO, PI and merged (AO/PI) channels at 10X objective magnification. Scale bar = 400 µm.

3.3 A.G. induces intracellular ROS generation in MCF-7 cells

Intracellular ROS levels, assessed using the DCFH-DA fluorescent probe, were significantly elevated in A.G. (100 µM)-treated MCF-7 cells compared with untreated control (Figure 3). Fluorescence microscopy showed markedly increased green DCF fluorescence intensity in A.G.-treated cells relative to control, and quantification of relative fluorescence intensity confirmed a statistically significant increase in intracellular ROS level in the A.G.-treated group ($p<0.001$ vs. control), supporting a pro-oxidant, ROS-mediated mechanism underlying the cytotoxic and apoptotic effects of A.G. observed in the MTT and AO/PI assays.

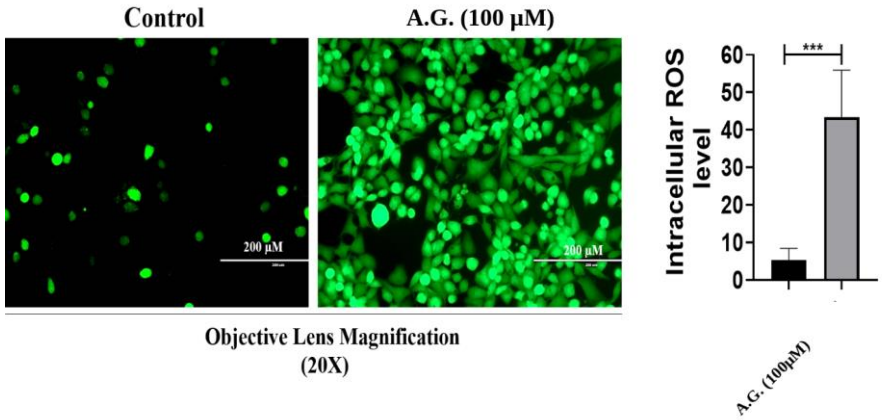

Figure 3. Intracellular ROS generation in MCF-7 cells following treatment with A.G. (100 µM) for 24 h, assessed by DCFH-DA fluorescence microscopy (20X objective magnification; scale bar = 200 µm) and quantified as relative intracellular ROS level. ***p<0.001 vs. control.

3.4 A.G. suppresses MCF-7 cell migration (wound-healing assay)

The anti-migratory effect of A.G. on MCF-7 cells was evaluated using the wound-healing scratch assay over 24 h (Figure 4). At 0 h, wound width was comparable between control and A.G. (100 µM)-treated groups. By 12 h, control cells had begun to migrate into the wound area and partially close the gap, whereas A.G.-treated cells showed visibly slower wound closure. This difference was more pronounced at 24 h, at which time control cells showed near-complete wound closure, while A.G.-treated cells retained a substantially wider open wound area, indicating that A.G. treatment significantly suppressed the migratory capacity of MCF-7 cells relative to untreated control.

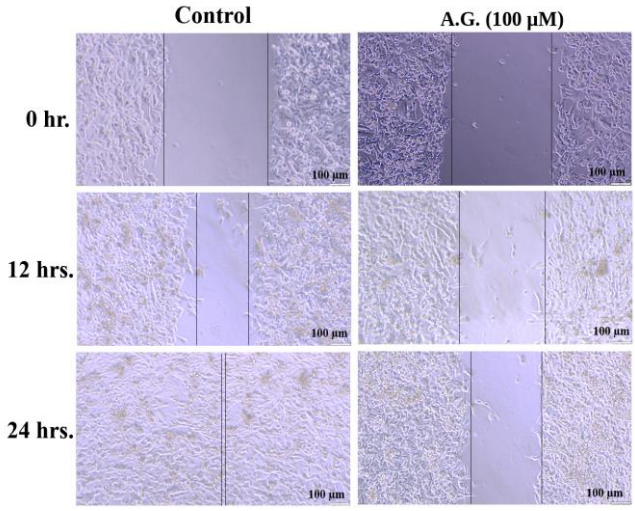

Figure 4. Representative phase-contrast images of the wound-healing (scratch) migration assay in MCF-7 cells, comparing control versus A.G. (100 µM)-treated cells at 0, 12 and 24 h. Scale bar = 100 µm.

4. Discussion

The present study demonstrates that a hydroalcoholic combination extract of Ashwagandha and green tea (A.G.) exerts concentration-dependent cytotoxicity, promotes apoptotic morphological changes, elevates intracellular ROS levels, and inhibits migration in MCF-7 human breast cancer cells, collectively supporting its potential as a multi-targeted phytotherapeutic anti-breast-cancer agent.

The concentration-dependent decline in cell viability observed in the MTT assay is consistent with previous reports describing anti-proliferative activity of Withania somnifera extracts and withanolides against MCF-7 cells, with reported IC50 values in the low-to-mid micromolar/microgram range depending on extract type and exposure duration [2,3,6]. Similarly, green tea extracts and EGCG have been independently shown to reduce MCF-7 viability and selectively target malignant over non-tumoral breast cells through p53-dependent mechanisms [9,11]. The IC50 of approximately 100 µM

observed for the A.G. combination in the present study falls within a pharmacologically plausible range for a combined botanical extract and is consistent with the possibility of an additive or synergistic interaction between the two component extracts, in line with prior work demonstrating enhanced antioxidant potential when Ashwagandha and green tea are combined [12].

The AO/PI staining results, showing a clear shift from green (viable) to orange-red (PI-positive) fluorescence following A.G. treatment, are characteristic of apoptotic and late apoptotic/secondary necrotic morphology and corroborate similar AO/PI-based observations reported for other Withania somnifera and green tea-derived agents in breast and other cancer cell lines [3,14,15]. This pattern is consistent with loss of plasma membrane asymmetry/integrity and nuclear condensation typically associated with programmed cell death.

A key mechanistic finding of this study is the significant elevation of intracellular ROS in A.G.-treated MCF-7 cells. Excessive ROS accumulation is a well-established trigger of the mitochondrial (intrinsic) apoptotic pathway, and withaferin A, the principal bioactive withanolide of Ashwagandha, has been specifically shown to induce ROS-mediated apoptosis in MCF-7 and MDA-MB-231 cells by inhibiting mitochondrial complex III activity, an effect attenuated by ectopic overexpression of Cu,Zn-superoxide dismutase [4,5]. Green tea polyphenols, including EGCG, have likewise been shown to elevate intracellular ROS and trigger mitochondrial-mediated apoptosis (chromatin condensation, loss of mitochondrial membrane potential, caspase-3/9 activation) in MCF-7 cells, an effect linked in part to modulation of the p53/Bcl-2 axis [16,17]. The ROS elevation observed with A.G. in the present study is therefore consistent with, and mechanistically links, both the cytotoxic (MTT) and apoptotic (AO/PI) findings reported here.

Finally, the suppression of MCF-7 migration in the wound-healing assay following A.G. treatment parallels previous reports that EGCG and green tea extracts inhibit breast cancer cell migration and invasion via downregulation of VASP/Rac1 activity and modulation of EGFR/CD44/EMMPRIN signalling networks [7,8,10]. Impaired migratory capacity is of particular translational relevance in breast cancer, where local invasion and metastatic spread are the principal determinants of clinical outcome; thus, the anti-migratory activity of A.G. observed here suggests a potential role beyond simple cytotoxicity, in restraining the invasive phenotype of breast cancer cells [18].

Taken together, these in vitro findings suggest that the combination of Ashwagandha and green tea in a single hydroalcoholic extract may offer a multi-pronged anti-breast-cancer effect, engaging cytotoxic, pro-apoptotic, pro-oxidant and anti-migratory mechanisms simultaneously. Nonetheless, this study has several limitations: it was restricted to a single cell line (MCF-7), a single combination ratio and a single high-dose time point (100 µM, 24 h) for the mechanistic assays (AO/PI, ROS, migration), and did not include molecular/protein-level confirmation of apoptotic pathway activation (e.g., caspase-3/9 activity, Bax/Bcl-2 expression) or in vivo validation. Future studies should include additional breast cancer cell lines (including triple-negative models such as MDA-MB-231), normal/non-tumorigenic breast epithelial cells as a selectivity control, dose- and time-course optimization of the mechanistic assays, molecular pathway analysis, and evaluation of the individual extracts alongside the combination to formally establish additive/synergistic interaction (e.g., by combination index analysis) [19,20].

5. Conclusion

The hydroalcoholic combination extract of Ashwagandha and green tea (A.G.) exhibited concentration-dependent cytotoxicity against MCF-7 human breast cancer cells, induced apoptotic morphological changes as evidenced by AO/PI staining, significantly elevated intracellular ROS levels, and suppressed cell migration in the wound-healing assay. These findings collectively indicate that A.G. exerts anti-breast-cancer activity through ROS-mediated apoptosis and anti-migratory mechanisms, supporting its further investigation as a candidate natural adjunct in breast cancer management.

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