Research Article

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EVALUATION OF *IN VITRO* ANTICANCER AND ANTIOXIDANT ACTIVITIES OF A NOVEL POLYHERBAL AYURVEDIC FORMULATION IN A549 LUNG ADENOCARCINOMA CELLS

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ABSTRACT

Introduction: Lung cancer remains a major cause of cancer-related mortality. Polyherbal Ayurvedic formulations may provide multi-component biological activity, but each formulation requires direct experimental evaluation. Materials and Methods: A twelve-plant formulation was tested. Cell viability was measured by a tetrazolium-based colorimetric assay, free-radical scavenging by a 2,2-diphenyl-1-picrylhydrazyl assay, and apoptosis-associated damage by deoxyribonucleic acid fragmentation. Peripheral blood mononuclear cells were included as a preliminary non-malignant comparator. Results: A549 viability declined concentration-dependently and reached approximately 30–35% of untreated control at 2500 µg/mL, whereas peripheral blood mononuclear cells retained comparatively high viability. Radical scavenging reached approximately 70% at the highest concentration. Treated A549 cells displayed low-molecular-weight deoxyribonucleic acid fragments, although fragmentation was less pronounced than with paclitaxel. Conclusion: The formulation demonstrated concentration-dependent cytotoxicity against A549 cells, substantial radical-scavenging activity, apoptosis-associated DNA fragmentation, and comparatively preserved peripheral blood mononuclear-cell viability, supporting further preclinical evaluation as a candidate polyherbal anticancer formulation.

Keywords: A549 cells, antioxidant activity, apoptosis, Ayurveda, lung cancer, polyherbal formulation

INTRODUCTION

Cancer is a complex, multifactorial disease and a major global health burden. Lung cancer remains one of the leading causes of cancer-related mortality worldwide.[1] Non-small cell lung cancer is the predominant histological group and continues to have an unfavorable prognosis despite advances in diagnosis and treatment.[2] Current management includes surgery, radiotherapy, cytotoxic chemotherapy, targeted therapy, and immunotherapy; however, treatment efficacy may be limited by adverse effects, disease recurrence, and the development of drug resistance.[3] These limitations support continued investigation of agents that act through complementary biological pathways while maintaining acceptable selectivity.

Ayurveda and other traditional systems of medicine have long used plant-derived preparations for complex and chronic conditions.[4] Polyherbal formulations combine multiple plant materials and may contain diverse classes of phytochemicals, including alkaloids, flavonoids, terpenoids, and polyphenols. Such preparations are proposed to influence multiple biological processes, including cell proliferation, oxidative stress, inflammation, and programmed cell death.[5] However, the activity of a combined formulation cannot be assumed from its individual ingredients and must be evaluated experimentally.

The formulation investigated in this study contains twelve medicinal plants: *Actiniopteris radiata*, *Myristica fragrans*, *Zanthoxylum alatum*, *Pluchea lanceolata*, *Nelumbo nucifera*, *Lepidium sativum*, *Myrica esculenta*, *Syzygium aromaticum*, *Hordeum vulgare*, *Vitex negundo*, *Solanum xanthocarpum*, and *Withania somnifera*. Several constituents have pharmacological properties relevant to cancer research. *Withania somnifera* contains withanolides, including withaferin A, that have been investigated in integrative oncology.[6] Neferine from *Nelumbo nucifera* has inhibited human lung cancer cell growth through mitogen-activated protein kinase signaling and cell-cycle arrest.[7] *Solanum xanthocarpum* is a source of steroidal glycoalkaloids.[8] Eugenol from *Syzygium aromaticum* has documented antioxidant, anti-inflammatory, and anticancer activities,[9] while glucosinolates from *Lepidium sativum* have shown cytotoxic and apoptosis-related effects in cancer cell models, including A549 cells.[10]

The present study assessed its effects on A549 cell viability, its selectivity relative to peripheral blood mononuclear cells, its chemical radical-scavenging capacity, and its association with DNA fragmentation. Together, these complementary endpoints were used to characterize the *in vitro* anticancer and antioxidant activity profile of the complete formulation.

MATERIALS AND METHODS

Plant material and formulation preparation

The polyherbal formulation consisted of *Actiniopteris radiata*, *Myristica fragrans*, *Zanthoxylum alatum*, *Pluchea lanceolata*, *Nelumbo nucifera*, *Lepidium sativum*, *Myrica esculenta*, *Syzygium aromaticum*, *Hordeum vulgare*, *Vitex negundo*, *Solanum xanthocarpum*, and *Withania somnifera*. A stock preparation was diluted in aqueous medium to obtain working

concentrations of 1, 10, 100, 1000, and 2500 µg/mL for the *in vitro* experiments.

Cell and culture conditions

The human lung adenocarcinoma cell line A549 and normal human peripheral blood mononuclear cells were used for cytotoxicity testing. A549 cells served as the cancer model, whereas peripheral blood mononuclear cells were used for a preliminary assessment of selectivity. Cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and were subcultured at 80–90% confluence. Comparable A549 culture and cytotoxicity procedures have been reported previously.[11]

Tetrazolium-based cytotoxicity assay

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.[12] A549 cells and peripheral blood mononuclear cells were seeded in 96-well plates at 5×10^3 cells per well and exposed to the formulation at 1, 10, 100, 1000, or 2500 µg/mL for 24 h. Untreated cells served as the negative control. After treatment, MTT solution (5 mg/mL) was added and the plates were incubated for 4 h at 37 °C. The medium was removed, and 100 µL acidified isopropanol containing 1 N hydrochloric acid was added to each well to dissolve the formazan crystals. Plates were shaken gently for 20 min, and absorbance was measured at 570 nm. Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = [\text{OD of treated cells} / \text{OD of untreated cells}] \times 100$$

Free-radical scavenging assay

Antioxidant capacity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay, adapted from the method of Blois and a subsequent plant-extract protocol.[13, 14] A 0.1 mM DPPH solution in methanol was prepared freshly and protected from light. The formulation was tested at 1-2500 µg/mL. For each reaction, 100 µL formulation solution was mixed with 100 µL DPPH solution and incubated in the dark at room temperature for 30 min. Ascorbic acid served as the positive control. Absorbance was measured at 517 nm, and radical-scavenging activity was calculated as follows:

$$\text{Radical-scavenging activity (\%)} = [(A_{control} - A_{sample}) / \text{Acontrol}] \times 100$$

where $A_{control}$ is the absorbance of the radical solution without the test sample and A_{sample} is the absorbance of the radical solution containing the formulation.

Deoxyribonucleic acid fragmentation assay

DNA fragmentation was examined as an indicator of apoptosis. A549 cells were seeded in six-well plates at 1×10^6 cells per well and treated for 24 h with the selected formulation concentration. Vehicle-treated cells served as the negative control, and paclitaxel served as the positive control. Cells were harvested, and genomic DNA was extracted using a dimethyl sulfoxide-sodium dodecyl sulfate-Tris-EDTA buffer procedure. Equal sample volumes (16 µL lysate) were loaded on a 2% agarose gel prepared in Tris-acetate-EDTA buffer containing ethidium bromide. Electrophoresis was performed at 80 V for approximately 2 h. DNA bands were visualized under ultraviolet illumination, and densitometric analysis was used to compare the distribution of high- and low-molecular-weight DNA. DNA-fragmentation and apoptosis analyses have been used in previous A549 studies.[15]

Statistical analysis

Experiments were performed in triplicate, and data were expressed as mean ± standard deviation. Graphical presentation and statistical analysis were performed using GraphPad Prism version 8.

Ethical approval

The study used established commercially available cell lines and did not involve the collection of human samples or direct participation of human subjects.

RESULTS

Cytotoxicity and selectivity

The formulation produced a concentration-dependent reduction in A549 cell viability after 24 h of exposure (Figure 1A). Viability at 1, 10, and 100 µg/mL remained close to that of untreated cells. A reduction was evident at 1000 µg/mL, and the greatest effect was observed at 2500 µg/mL, where approximately 30–35% of control viability remained. In contrast, peripheral blood mononuclear-cell viability remained close to control values across the tested concentration range, including at 2500 µg/mL.

Morphological assessment

Microscopic examination supported the concentration-dependent pattern observed in the MTT assay (Figure 1B). Untreated A549 cells showed an adherent, elongated morphology and high cell density. Cell density remained broadly similar from 1 to 100 µg/mL. At 1000 µg/mL, fewer cells were visible and rounding and detachment were more evident. At 2500 µg/mL, cell density was markedly reduced, and the remaining cells were predominantly rounded and non-adherent.

Free-radical scavenging activity

The formulation showed a concentration-dependent increase in DPPH radical-scavenging activity (Figure 2). Activity was low at the lower concentrations and increased progressively with concentration. At 2500 µg/mL, the formulation achieved approximately 70% scavenging, approaching the activity of ascorbic acid under the conditions shown in the graph.

Deoxyribonucleic acid fragmentation

The untreated control showed predominantly intact high-molecular-weight genomic DNA. Paclitaxel treatment produced a more extensive fragmentation pattern. The formulation-treated sample showed reduced high-molecular-weight DNA intensity and detectable lower-molecular-weight fragments. The densitometric profiles similarly showed redistribution of signal toward the lower-molecular-weight region in the treated sample compared with the control. These findings are consistent with apoptosis-associated DNA fragmentation, although the pattern was less pronounced than that of the paclitaxel control (Figure 3).

DISCUSSION

The present study demonstrates a coherent *in vitro* activity profile for the twelve-plant Ayurvedic formulation in A549 lung adenocarcinoma cells. The concentration-dependent reduction in A549 viability, with approximately 30-35% viability remaining at 2500 µg/mL, was supported by the corresponding loss of cell density, rounding, and detachment, while peripheral blood mononuclear cells retained comparatively high viability, suggesting a favorable preliminary differential response between malignant and non-malignant cells. This overall pattern is consistent with previous reports of anticancer activity from traditional polyherbal preparations in non-small-cell lung cancer models[2] and with apoptosis-associated growth inhibition reported in A549 cells using plant-derived preparations such as

theabrownin.[15] The lower-molecular-weight DNA fragments observed after treatment further support an apoptosis-associated response; apoptosis is a well-established programmed cell-death pathway in cancer biology,[16] and the biological plausibility of this finding is strengthened by reported anticancer actions of phytochemicals relevant to the formulation, including withaferin A[17] and solamargine,[18] as well as by A549 studies showing extrinsic/intrinsic apoptotic signaling with *Calotropis gigantea* extract,[19] p53-mediated cell-cycle arrest and apoptosis with Renshenwuweizi decoction,[20] and caspase-9/mitochondrial reactive-oxygen-species-associated apoptosis with plumbagin.[21] The approximately 70% DPPH radical-scavenging activity at the highest concentration also indicates substantial chemical antioxidant capacity, consistent with the established association between phenolic/flavonoid constituents and radical-scavenging activity[22] and with the antioxidant properties reported for clove-derived constituents such as eugenol.[23] At the same time, redox-active phytochemicals may exert context-dependent effects in cancer cells, and withaferin A has been shown to induce oxidative-stress-mediated apoptosis and DNA damage in cancer models,[24] providing a plausible biological framework in which radical-scavenging capacity and anticancer activity can coexist. Taken together, the concordant cytotoxic, morphological, radical-scavenging, and DNA-fragmentation findings support meaningful biological activity of the complete formulation rather than of any single constituent. Because the study evaluated the formulation as a whole, the results are most appropriately interpreted at the formulation level without inferring pharmacological synergy. Although the strongest cytotoxic effect occurred at the highest tested concentrations and mechanistic confirmation beyond DNA fragmentation remains a logical next step, these considerations define the next phase of characterization rather than detract from the observed response. Overall, the data support the formulation as a promising candidate for expanded preclinical evaluation, including quantitative potency and selectivity assessment, orthogonal apoptosis assays, phytochemical standardization, and validation in additional lung cancer and non-malignant lung models.

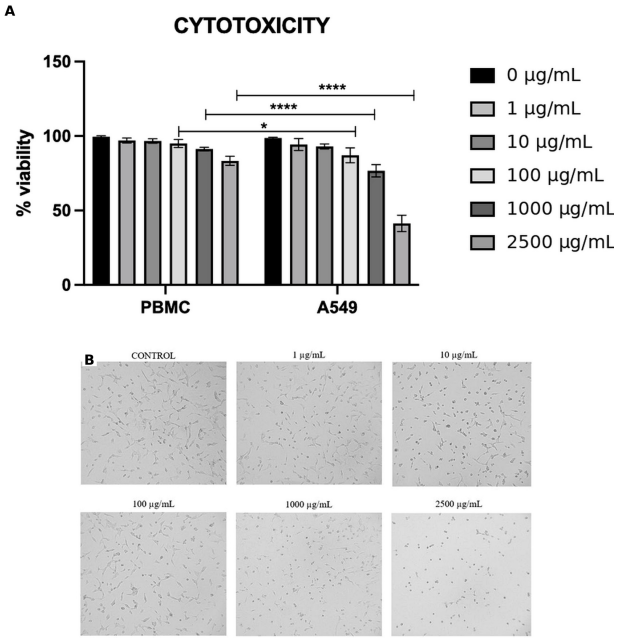

Figure 1: Effect of the polyherbal formulation on A549 cells and peripheral blood mononuclear cells.
A: Cell viability after 24 h exposure to 0–2500 µg/mL. B: Representative A549 morphology after 24 h exposure. Data in panel A are presented as mean ± standard deviation (n = 3).

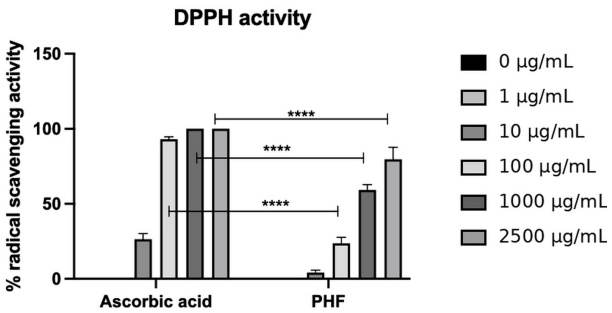

Figure 2: Free-radical scavenging activity of the polyherbal formulation and ascorbic acid.
PHF = Polyherbal formulation. Data are presented as mean ± standard deviation (n = 3).

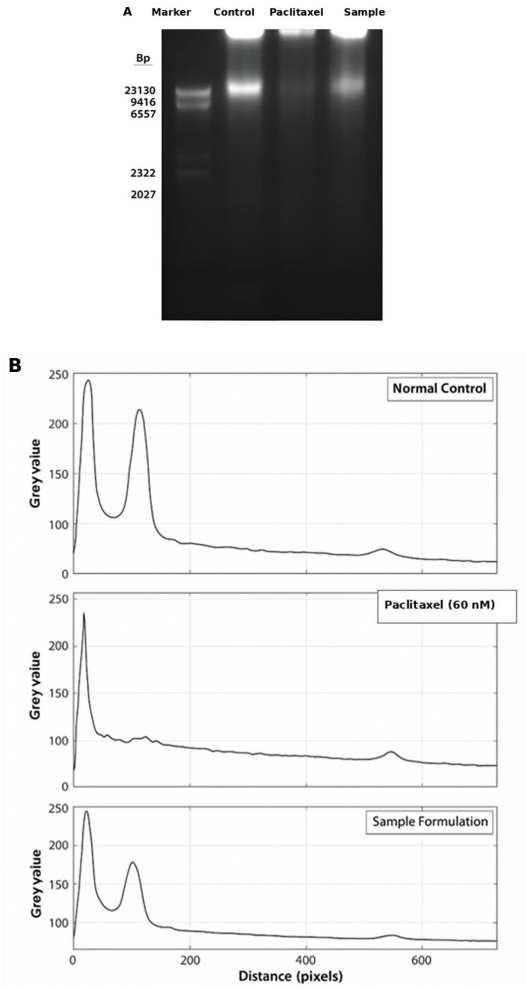

Figure 3: Deoxyribonucleic acid fragmentation in A549 cells.
A: Agarose-gel electrophoresis of molecular-weight marker, untreated control, paclitaxel positive control, and formulation-treated sample.

CONCLUSION

The twelve-plant polyherbal formulation demonstrated concentration-dependent cytotoxic activity against A549 lung adenocarcinoma cells, together with comparatively preserved peripheral blood mononuclear-cell viability, substantial DPPH radical-scavenging activity, and DNA fragmentation consistent with an apoptosis-associated response. Together, these findings establish a clear *in vitro* anticancer and antioxidant activity profile and support the formulation as a promising candidate for further preclinical development. Future studies can build on these findings by defining quantitative potency and selectivity, confirming molecular pathways of cell death, standardizing the phytochemical profile, and evaluating activity in additional lung cancer models and *in vivo* systems.

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