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ANTI PROLIFERATIVE ACTIVITY OF *ANDROGRAPHIS PANICULATA* WHOLE PLANT EXTRACT ON A549 LUNG CANCER CELL LINE.

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ABSTRACT

Introduction: Lung cancer is one of the most common forms of cancer in the world. Cases are increasing globally everyday mainly due to increased habits of tobacco smoking. Timely diagnosis of lung cancer is complicated and requires effective coordination between multiple disciplines. Treatment modalities require a more personalised approach as lung cancer is a heterogeneous disease influenced by multiple factors. *Andrographispaniculata*is a medicinal plant which is traditionally used for many centuries for various purposes like upper respiratory tract infections, viral infections, etc. It is widely used in the field of Unani and Ayurveda.

Aim: The aim of our study is to find out whether *Andrographispaniculata*whole plant extract has anti-proliferative activity on A549 lung cancer cell line.

Materials and Methods: The cells (1×10^5 cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with replications. Treatment was conducted for 24hrs with different concentrations (10, 20, 40, 80, 160, 320 µg/ml) of andrographolide. After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilised with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance in control cells (0.1 % DMSO) (A570). The IC50 was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. Percentage of cell viability was calculated following the equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Inhibition (%)= 100- cell viability (%)

Results and Discussion:*Andrographispaniculata*whole plant extract was found to have anti proliferative activity against A549 lung cancer cell line. We observed that when the concentration of plant extract increases the rate of cell death also increases, which clearly shows the anticancer efficacy of the plant extract.

Conclusion: Hence *Andrographispaniculata*can be used as an effective treatment against lung cancer. Further investigation at pre-clinical and clinical levels for establishing it as a potential agent for cancer therapy.

Key words: Lung cancer, A549, *Andrographispaniculata*, cytotoxic, antiproliferative.

INTRODUCTION

Lung cancer is the most common form of cancer in the world (12.3% of all cancers), with an estimated 1.2 million new cases in 2000. Tobacco smoking is the most important cause of lung cancers with 80%–90% arising in cigarette smokers (1). Unfortunately, owing to the global increase in the number of smokers since 1980,2 the burden of lung cancer will likely continue to increase in the coming years primarily in developing countries, where high-quality cancer registry data are unavailable (2). Though lung cancer is strongly associated with cigarette smoking there is a substantial minority of

patients who have never smoked. In this case, the population has more distinct molecular markers and less established risk factors (3).

Lung cancer is relatively rare before the fifth decade of life; risk increases with age thereafter. Men are more affected than women. Coming into clinical manifestations, dyspnea is present in one-third to one-half of patients, there is risk of developing pulmonary emboli, pneumothoraces, pleural effusions, and pericardial effusions, chest pain is a less common symptom, paraneoplastic syndromes, Lambert-Eaton myasthenic syndrome. Cerebellar ataxia, hypercalcemia, etc(4). Timely detection, diagnosis, and subsequent treatment for lung cancer is critical to patient outcomes and well- being. Delays in any part of the process, from initial evaluation and referral, to definitive diagnosis, treatment, follow-up, and survivorship care, may lead to adverse patient outcomes.

Timely lung cancer diagnosis and treatment requires quick and effective coordination and communication across multiple disciplines—e.g., primary care, radiology, pulmonology, medical oncology, radiation oncology, and surgery (5). Lung cancer is a very heterogeneous disease, at a cellular and histological level. Diagnostic challenges are deeply related to the development of personalized therapy and molecular and precise histological characterizations of lung cancer (6). Targeted therapy and immunotherapy for lung cancer include targeting of epidermal growth factor mutations, EML4-ALK Translocations, ROS1 rearrangements, BRAF V600 mutations, MET Amplification, RET rearrangements, HER2 mutations, etc. (7).

Andrographispaniculata (AP) is a medicinal plant traditionally used as an anti-inflammatory and antibacterial herb. Andrographolide, the major active component of A. paniculata, exhibits diverse pharmacological activities, including anti-inflammation, anti- cancer, anti-obesity, anti-diabetes, and other activities. (8). In the Unani and Ayurvedic medicines, AP is one of the most used medicinal plants. It is commonly called 'Nilavembu'. The balancing of proinflammatory and anti-inflammatory cytokines is the result of anti-inflammatory performance of andrographolide. (9). AP has been found to counteract interference with the cell cycle. Such interference is the basis for the development of cancer or infection with viruses such as HIV-1. Andrographolides are thought to enhance immune system functions such as production of white blood cells (scavengers of bacteria and other foreign matter), release of interferon, and activity of the lymph system. (10)Our team has extensive knowledge and research experience that has translate into high quality publications(11–15),(16),(17),(18),(19),(20),(21),((13,22,23),(24–28) .,(29),(30)

The aim of this study is to find the anti proliferative activity of Andrographispaniculata whole plant extract on A549 lung cancer cell line.

MATERIALS AND METHODS

Chemicals

DMEM medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO were from Himedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sisco Research Laboratories (SRL) India.

Preparation of the Herbal Extract

Stem powder of Andrographispaniculata (AP) was obtained from IMPCOPS (Chennai, India) and used for the present study. About 50 g of AP powder was soaked in 500 mL of aqueous and kept for 3 days in a static condition at room temperature. The solution was then filtered with crude filter paper followed by whatmann paper. Fine filtrate was subjected to rota evaporation after that 3g of the material was obtained. The total ethanol extract was concentrated in a vacuum evaporate and immediately stored at 4℃.

Cell culture reagents Dulbecco's Modified Eagle's medium(DMEM)

Commercially available DMEM contains 7.5% sodium bicarbonate solution. 5ml of penicillin/streptomycin solution and 0.5ml of amphotericin B solution were added to 500ml of DMEM. The medium was then placed inside the hood and sterile filtered (0.22µ). The medium was dispensed into sterile containers and stored at 4°C.

Growth Medium [DMEM with 10% Fetal Bovine Serum (FBS)]

10ml of FBS was made up to 100ml using sterile DMEM. It was stored in a sterile container in cool and aseptic condition.

Phosphate Buffered Saline (PBS; pH 7.4)

0.63 g of sodium phosphate monobasic (NaH_2PO_4), 0.17 g of sodium phosphate dibasic (Na_2HPO_4) and 4.5 g of sodium chloride (NaCl) were dissolved in 500 ml of double autoclaved milliQ water. Using 1 N HCl and 1 N NaOH, the pH was adjusted to 7.4, sterile filtered (0.22 µ) and then stored in a sterile container.

Trypsin-EDTA

Trypsin was purchased as 1 x with EDTA (0.5% trypsin, 5.3 mM EDTA sodium salt). (Note: Freeze-thaw process does not affect the enzyme activity. Thawing is done at room temperature).

Cell Line

Human lung adenocarcinoma-A549 cell lines were obtained from the National Centre for Cell Science (NCCS, Pune), India. The cells were cultivated in T255 culture flasks which contained DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using Trypsin-EDTA solution.

Cell proliferation (MTT) Assay

The proliferation of A549 cells were evaluated by MTT assay Koka et al., 2018(31).The anticancer activity of different concentrations of *Andrographispaniculata*whole plant extract on A549 were determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay to assess the cytotoxicity according to the method described by Koka P et al, 2018. For the assay, cells were plated in 96-well plates at the density of $5x10^3$/100µl. The cells were incubated for 24 hrs. The cells were then treated with AP extract at increasing concentrations of 10, 20, 40, 80, 160 and 320µg/ml. Wells with extract free mediums were used as negative controls. After 24 hrs of incubation at 37°C , 10µl MTT reagent was added to each well and incubated for 4 hrs in dark at 37°C. 100µl of Sorenson glycine buffer comprising of 0.1M glycine, 0.1M NaCl, pH 10.5 with 0.1N NaOH was added to the wells in order to solubilize the formazan crystals formed by the viable cells. The absorbance was then measured at 570 nm. The experiment was repeated thrice and each concentration of extract was tested in triplicates. The percentage of cell viability and inhibition percentage were calculated.

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Inhibition (%) = 100 - Cell Viability (%)

Statistical Analysis

All data obtained were analyzed by Student-t-test using MS-Excel, represented as mean ± SD for six animals in each group. The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. In all tests, the level of statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

From fig 1 we observed that the dose dependent manner *Andrographispaniculata*whole plant extract treatment significantly decreases the cell viability in dose and time dependent manner. The antiproliferative activity of *A.paniculata* in lung cancer cells at 20µg/ml after 24hrs of incubation, has inhibited the 50% of cell proliferation, and it has been considered as IC_{50} value respectively.

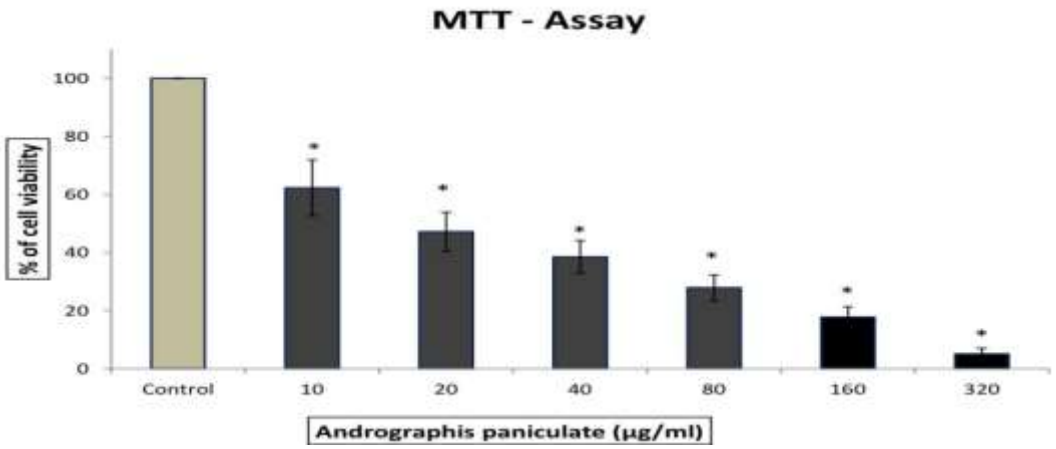

Fig 1: Graph showing concentration of *Andrographispaniculata*whole plant extract on x axis and percentage of cell viability on y axis. We infer that as the concentration of plant extract increases, the percentage of cell viability decreases linearly.

Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

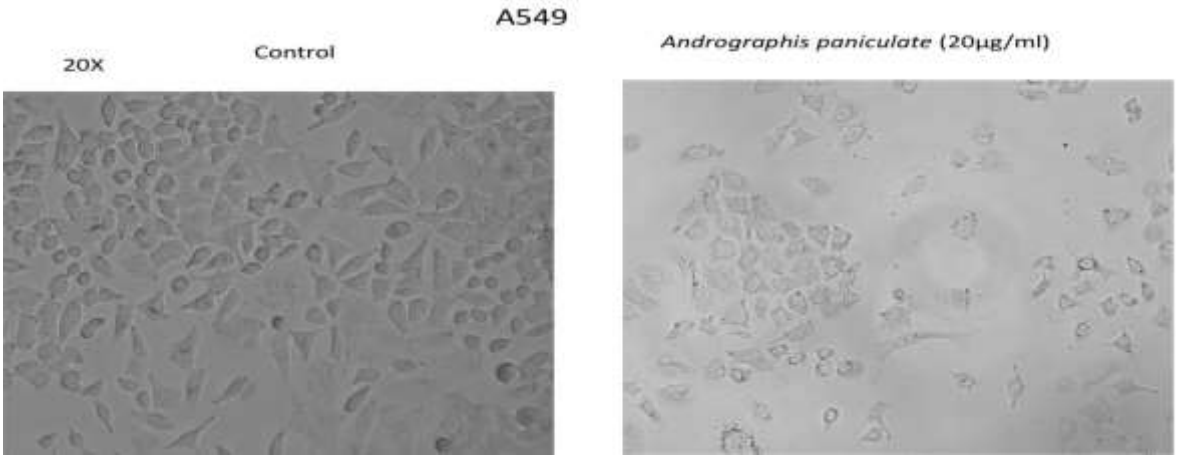

Fig 2: Assessment of cell morphology of A549 treated with andrographolidepaniculataas compared to control. Images were obtained from inverted phase contrast microscopy with 20x magnification.

Uncontrolled proliferation of cells due to loss of cell cycle control pathways leads to cancer. Cancer cells display much higher rates of proliferation than normal cells. Some currently used antitumor drugs, such as vinca alkaloids and taxanes, act by targeting microtubules and inhibiting mitosis. However these inhibitors have still not been approved for use in chemotherapy regimens. (32) Chemotherapy administration may result in the disruption of circadian rhythms and impairment of quality of life (QoL) of cancer patients. (33)

Medicinal plants are an integral part of human life to combat the sufferings from the dawn of civilization. It is estimated that more than 80,000 of total plant species have been identified and used as medicinal plants around the world. Andrographispaniculata (AP) is an important medicinal plant and widely used around the world. It belongs to the family Acanthaceae. Its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides.(9) It was found that the aqueous extract showed significant antimicrobial activity, which may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides. (34)Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers. (35)The compound exerts direct anticancer activity on cancer cells by cell-cycle arrest at G0/G1 phase through induction of cell-cycle inhibitory protein p27 and decreased expression of cyclin-dependent kinase 4 (CDK4). (36). Andrographolide and its derivatives have been shown to have anti-inflammatory effects in experimental models of asthma, stroke and arthritis, as well as in patients with upper respiratory tract infections. Andrographolide reduces the production of cytokines, chemokines, adhesion molecules, nitric oxide and lipid mediators, probably via inhibition of the nuclear factor (NF)-κBsignalling pathway. (37)Andro induced apoptosis in human cancer cells via activation of caspase 8 in the extrinsic death receptor pathway and subsequently with the participation of mitochondria.(38)Andro triggered a caspase 8-dependent Bid cleavage, followed by a series of sequential events including Bax conformational change and mitochondrial translocation, cytochrome c release from mitochondria, and activation of caspase 9 and 3. Hence, it was found that pro-apoptotic Bcl-2 family members (Bid and Bax) are the key mediators in relaying the cell death signaling initiated by Andro. (39)
In recent years, pharmaceutical chemists have synthesized numerous andrographolide derivatives, which exhibit essential pharmacological activities such as those that are anti-inflammatory, antibacterial, antitumor, antidiabetic, anti-HIV, antifeedant, and antiviral. (38,40)In a radiation therapy study, andrographolide was found to sensitize Ras-transformed cells and significantly delay tumor growth. Experimental evidence suggests that andrographolide attenuates endothelial cell motility and tumor-endothelial cell interaction.(39,41).

From our results*Andrographispaniculata*whole plant extract induces apoptosis thereby inhibiting the cell proliferation against lung cancer cell lines. It was found that the apoptotic activity was dose dependent and cell viability has reduced accordingly at the concentration of 20µg/ml 50% of the cell proliferation has inhibited for 24hrs.

CONCLUSION

The present study clearly showed that the AP whole plant extract has enhanced antiproliferative activity in A549 lung cancer cell lines. Within the limitations of this study it was found that *Andrographispaniculata*whole plant extract was cytotoxic and it inhibited the cell proliferation against A459 lung cancer cell line. With further research on AP we can find out whether it can be used as an early and effective natural treatment for lung cancer in future medicine. However, the in vivo study using an experimental cancer animal model needed to prove a potential anticancer effect of *Andrographispaniculata.*

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CONFLICT OF INTEREST

The author declares that there was no conflict of interest in the present study.

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