

Antiproliferative efficacy of *Centella asiatica* aqueous leaf extract on human lung cancer cell line.

ABSTRACT :

AIM: To assess the anti proliferative efficacy of *Centella asiatica* aqueous leaf extract on human lung cancer cell line

INTRODUCTION : *Centella asiatica* (family Apiaceae), commonly known as Indian Pennywort, is an ethnomedical plant that is widely used in India for treating skin problems and for revitalizing the brain and nervous system . It has been reported to have various pharmacological activities, including antioxidant, anti-inflammatory, anticancer, neuroprotective, cardioprotective, skin protective, radioprotective, immunomodulatory, memory-enhancing, and wound-healing properties. Asiatic acid and its derivatives possess a broad spectrum of pharmacological activities, such as anticancer, wound healing, anti-inflammatory, antidiabetic ,hepatoprotective and neuroprotective activities. The aim of the study is to assess the antiproliferative efficacy of *Centella asiatica* aqueous leaf extract on human lung cancer cell line.

MATERIALS AND METHODS : Human lung adenocarcinoma-A549 cell line was procured from National Centre for Cell Science (NCCS, Pune), India.

The effect of *Centella asiatica* on cell viability was measured by MTT assay following the method by Mosmann Briefly, the cells (1×10^5 cells per ml) were seeded in a 96 well micro titre plate (100 μ l per well) with replications. Treatment was conducted for 24 with different concentrations (50,100,150,200,250 and 300 μ M) of *Centella asiatica* .After incubation, 20 μ l 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 solubilized 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. Percent cell viability was calculated following the equation: Cell viability (%) = $\{A570_{od} \text{ of (sample)}/A570_{od} \text{ of (control)}\} \times 100$

RESULTS AND DISCUSSION: Results show that *centella asiatica* has an antiproliferative effect on human lung cancer cell line. The characterisation of morphological changes in human lung cancer cells treated with *Centella asiatica* leaf extract compared to their respective controls were observed under phase contrast microscope. Visible destruction of the cancer cells were observed at the initial concentration of 25 microgram/ml. Further concentrations however showed increased antiproliferative activity proving it to be a potent anticancer drug.

CONCLUSION: This research concludes that centella asiatica leaf extract has a potent antiproliferative effect on human lung cancer cell lines. However further research is needed to understand the mechanisms of cytotoxicity of the plants.

INTRODUCTION:

Lung cancer is the most common type of cancer and is associated with a high rate of cancer-related mortality worldwide. Lung cancer is generally divided into two major subtypes: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (1). Non-small cell lung cancer (NSCLC) types, including adenocarcinoma, squamous cell carcinoma and large cell carcinoma, account for more than 80% of total pulmonary malignancies (2). Although drugs targeting epidermal growth factor receptor (EGFR) mutations, such as gefitinib (Iressa®) and erlotinib (Tarceva®), have been approved for treatment of advanced NSCLC, their efficacy is limited, and more than 50% of these patients are not suitable for erlotinib and gefitinib treatment (3). Furthermore, almost all patients initially sensitive to gefitinib develop resistance to the drug (4). Therefore, it is essential to identify novel anticancer drugs or reagents against NSCLC. Centella asiatica (family Apiaceae), commonly known as Indian Pennywort, is an ethnomedical plant that is widely used in India for treating skin problems and for revitalizing the brain and nervous system (5). It has been reported to have various pharmacological activities, including antioxidant, anti-inflammatory (6), anticancer (7), neuroprotective, cardioprotective, skin, radioprotective, immunomodulatory, memory-enhancing, and wound-healing properties (8)(9). Studies on the chemical constituents of *C. asiatica* showed the presence of pentacyclic triterpenoids known as centelloids. These compounds are composed of terpene acids along with glycosides, including asiatic acid, asiaticoside, madecassic acid, madecassoside, brahmic acid, brahmoside, brahminoside, thankinaside, isothankuniside, madasiatic acid, centic acid, centelloside, and cenellic acid (10).

Asiatic acid and its derivatives possess a broad spectrum of pharmacological activities, such as anticancer, wound healing, anti-inflammatory (11), antidiabetic (12), hepatoprotective and neuroprotective activities. Asiatic acid (AA) is a triterpene extracted from *Centella asiatica* (L.) Urban (Umbelliferae) that has a long history of successful use in both traditional Chinese and Indian Ayurvedic medicine. Previous studies have demonstrated that AA exhibits a variety of pharmacological effects not only as an antioxidant (13), antiinflammatory and neuroprotective agent but also against cancer (14). Medicinal herbs are a rich source of various bioactive compounds, which have long been used in the treatment of many diseases, including cancer. Recently, compounds isolated from medicinal herbs (15) have been identified as potent radiotherapy enhancers, such as radiosensitizers. Asiatic acid, asiaticoside, and madecassic acid have been reported as the major components of *C. asiatica* and these compounds have exhibited anticancer activity in various cancer cell lines. Asiatic acid has showed an antiproliferative effect by regulating apoptosis in a variety of human cancer cells, such as breast cancer, lung cancer, and melanoma cells (16). Our team has extensive knowledge and research experience that has translate into high quality publications(17–21),(22),(23),(24)(25),(26),(27),(19,28,29),(30–34).,(35),(36)

MATERIALS AND METHODS

CELL PROLIFERATION ASSAY

The effect of *Centella asiatica* on cell viability was measured by MTT assay following the method by Mosmann (37)

Principle

The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in colour. Then the cells are lysed using 20% SDS solution, which release the formazan crystal. These crystals are solubilized by DMF present in the solubilizer. The colour developed is then determined in an ELISA reader at 620 nm.

Reagents

1. MTT [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide]: 0.5 mg MTT/ml of serum-free DMEM.
2. Solubilization solution: 20% w/v SDS in 50% of Dimethyl formamide.
3. Phosphate Buffered Saline (PBS; pH 7.4).

Procedure

Briefly, the cells (1×10^5 cells per ml) were seeded in a 96 well micro titre plate (100 μ l per well) with replications. Treatment was conducted for 24 with different concentrations (50,100,150,200,250 and 300 μ M) of *Centella asiatica*. After incubation, 20 μ l 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 solubilized 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. Percent cell viability was

calculated following the equation: Cell viability (%) = {A570od of (sample)/A570 od of (control)} x 100

Spectrophotometrical absorbance of the purple blue formazan dye was measured in microplate reader at 620 nm. The OD of each sample was then compared with the control OD and the graph was plotted.

STATISTICAL ANALYSIS

All data obtained were analyzed by Students-t-test using MS-Excel, represented as mean \pm 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 :

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Although a number of drugs targeting EGFR mutations have been developed to date, most advanced cases are still incurable. Medicinal herbs are a rich source of various bioactive compounds, which have long been used in the treatment of many diseases, including cancer . Asiatic acid , asiaticoside and madecassic acid have been reported as the major components of *C. asiatica* (38) and these compounds have exhibited anticancer activity in various cancer cell lines. Asiatic acid has showed an antiproliferative effect by regulating apoptosis in a variety of human cancer cells, such as breast cancer, lung cancer, and melanoma cells(33) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52). The characterisation of morphological changes in human lung cancer cells treated with *Centella asiatica* leaf extract compared to their respective

controls were observed under phase contrast microscope. Visible destruction of the cancer cells were observed at the initial concentration of 25 microgram/ml. Fig.1 shows the antiproliferative effect of centella asiatica as there is clear difference between the control cells and the destruction of A549 Cells that are treated with C.asiatica extract. The cells were treated with different concentrations such as 50, 100,150, 200, 250 and 300 $\mu\text{g}/\text{ml}$. As the concentration increased, the % of cell viability decreased gradually (fig.2) and showed increased antiproliferative activity proving it to be a potent anticancer drug.

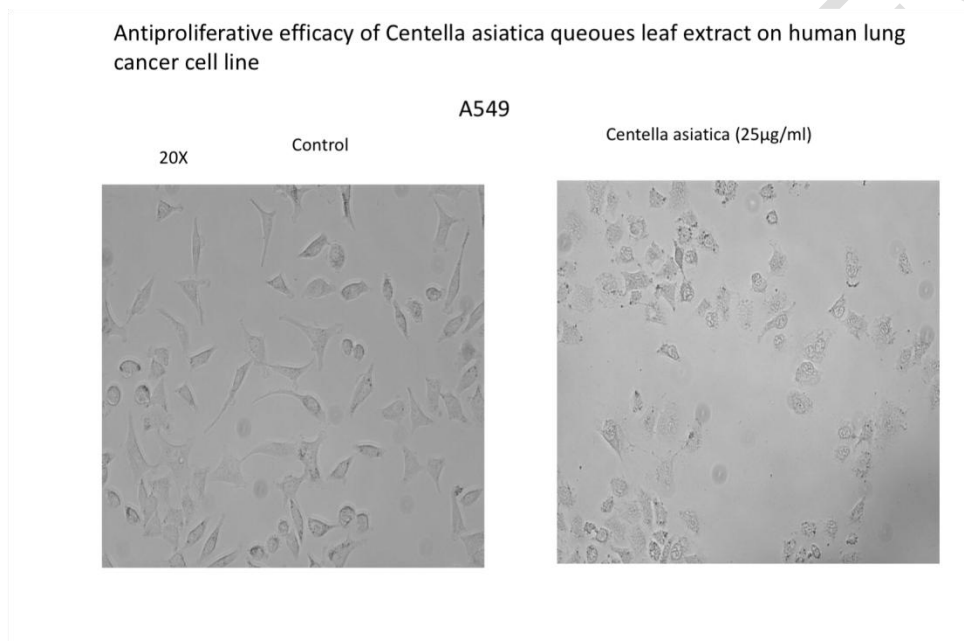


Fig.1 shows the phase contrast microscopy of control cells and experimental A549 cells.

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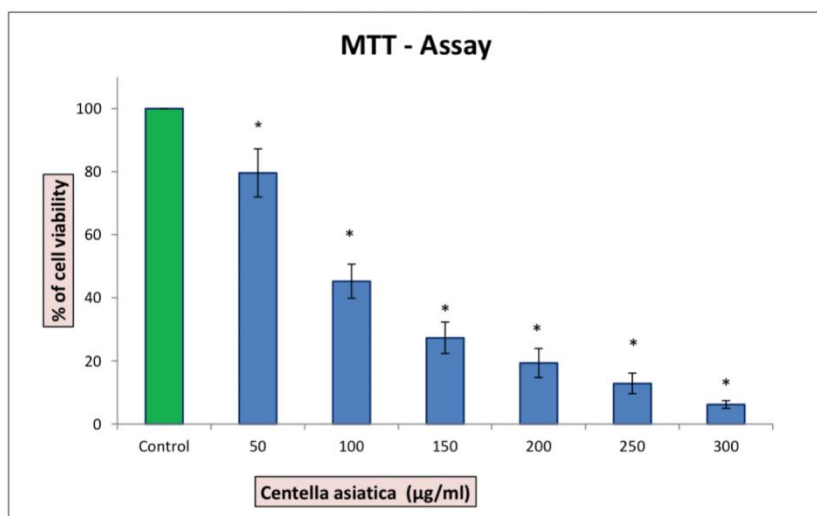


Fig.2 Graph shows the antiproliferative activity of *Centella asiatica* where X axis represents concentration of *C. asiatica* extract and Y axis represents % of cell visibility.

CONCLUSION :

This research concludes that *centella asiatica* leaf extract has a potent antiproliferative effect on human lung cancer cell lines. However further research is needed to understand the mechanisms of cytotoxicity of the plants and fluorescent microscopy can be used distinguish between viable and non viable cells more clearly.

NOTE:

The study highlights the efficacy of "AYURVEDA" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW