

# *In-vitro* Anticancer Activity of *Cardiospermum Halicacabum* against Human A549 Lung Cancer Cell

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## ABSTRACT

**Introduction:** Lung cancer is the major type of cancer that is developing all over the world. Approximately 1.8million new people were affected by lung cancer in 2012 among which 1.6million people were fatal. *Cardiospermum halicacabum* is also known as balloon vine, climber plant. It belongs to order sapindaceae and it has a high medicinal value.

**AIM:** To assess the anticancer activity of *Cardiospermum halicacabum* against human A549 lung cancer cell line.

**Materials and method:** The anticancer effect of *cardiospermum halicacabum* on A549 was measured by MTT assay and the cell morphological evaluation done by inverted phase contrast microscopy.

**Results and Discussion:** From the MTT assay plant extract of *Cardiospermum halicacabum* showed approximately 50% cell inhibition at 30 $\mu$ l/ mg concentration after 24hrs of treatment. Further it was confirmed with morphological evaluation using phase contrast microscopy. Overall, the MTT result clearly shows that the *C.halicacabum* has significantly reduced the cell viability in dose dependent manner effect in cell proliferation.

**Conclusion:** From the results, the extracts were cytotoxic to the lung cancer cells at 30 $\mu$ l/ mg concentration and taken as (IC50, $\mu$ l/ mg) value for further experiments. However more research is needed to understand the mechanisms of cytotoxicity of the plants.

**Key words:** *Cardiospermum halicacabum*, Lung cancer, Anticancer activity, Innovative techniques, Innovative technology

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## INTRODUCTION

Lung cancer is the leading cause of death in the 20th century. In India, the first primary cause of cancer driven death in men is lung cancer whereas it's second in case of women [1]. Approximately 1.8million new people were affected by lung cancer in 2012 among which 1.6million people were affected. 15% of the smokers who were suffering from lung cancer had traces of genetic susceptibility towards this disorder. There are various occupational hazards which cause the occurrence of lung cancer like asbestos exposure, which increases the rate of malignancy. Presence of general family history with cancer incidence, presence of chronic obstructive lung diseases may also lead to the occurrence of this disorder [2].

There exists a separate category of never makers who use substances other than tobacco like marijuana, e cigarette, etc. which is considered as the seventh most common cause of lung cancer, worldwide. The prevention measures of further invasions and maintenance of these disorders include limited exposure to air pollution, healthy body weight, exercise, healthy diet, protection from unknown carcinogens from inhalation. Despite numerous advances,

the mortality rate from lung cancer has been increasing day by day [3]. There are studies which concentrate on various treatment options to this disorder. Stage 1 pre-operative chemotherapy, surgery, high dose stereotactic body radiations. If the disorder gets locally advanced, 6 weeks of course thoracic radiotherapy is proceeded [4]. The last advanced lung cancer can be treated with molecular targeted therapy like EGFR mutations, genetic alterations and immunotherapy like immune system action suppression etc. which helps maintain and prevent further progression of the lung cancer [5].

*Cardiospermum halicacabum* belongs to the family sapindaceae. It grows in tropical and subtropical regions and is commonly called as balloon vines. It is mainly used for cough, nerve illness, arthritis, antioxidants, rubefacient and it contains antifungal properties [6]. *Cardiospermum halicacabum* is used in chinese medicine, it is a perennial plant where the leaves are compound and leaflets are membranous. It contains pyriform capsules that are wrangled. This plant is used as a traditional medicine and is used to cure many diseases [7].The inhibitor activity occurs through blocking the ion-ion chelating activity by beta-carotene, a fundamental component of the linolelate model system and by the inhibition of the radical DPPH [8].

Based on the previous study done, *Cardiospermum* and cyclophosphamide dose was injected into mice. The CTX administration caused myelosuppression and it caused decreased WBC count and the bone marrow cellularity [9]. While the hepatoprotective properties of the extract can aid in CCl<sub>4</sub>-induced liver injury, it also acts as a useful tool for the study of phenolic components [10]. Many researchers reported that evaluating plant derived compounds cytotoxicity, antioxidant activity and antipyretic activity has adjuvant therapy for human diseases as future medicine, [11]. The plant extract had an antihyperglycemic effect on streptozotocin-induced diabetic rats, where plasma and tissue glycoprotein levels were raised, and treatment of the plant extract restored normal levels [11,12]. Further, Rao's investigation discovered the ethanolic extract of the *Cardiospermum* plant has antidiarrheal properties. [11–13]. Our team has extensive knowledge and research experience that has translate into high quality publications [14-33]. The present study aims to evaluate the in vitro anticancer activity of *Cardiospermum halicacabum* against human 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

Whole plant powder of *Cardiospermum halicacabum* commercially purchased from IMPCOPS( Chennai, India) was used for the present study. About 150g of the plant powder was soaked in 500ml of aqueous/ 95% ethanol and kept for 3 days in a static condition at room temperature. The solution was filtered with a whatman filter paper preceded by filtration with crude filter paper. Fine filtrate was subjected to rota evaporation after that 3g of the material was obtained. The total ethanolic extract was concentrated using a vacuum evaporator and immediately stored at 40.

### Cell culture reagents

Commercially available Dulbecco's Modified Eagle's medium (DMEM) contains 7.5% sodium bicarbonate solution. To 500ml of DMEM, 5ml of penicillin/streptomycin solution and 0.5ml of amphotericin B solution was added. Then the medium was sterile filtered (0.22 $\mu$ ) inside the hood. The medium was then dispensed into a sterile container and stored at 4o.

### Growth medium (DMEM with 10% 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)

0.63g of sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>), 0.17g of sodium phosphate dibasic (NaHPO<sub>4</sub>) and 4.5g of sodium chloride (NaCl) were dissolved in 500ml of double autoclaved milliQ water. The pH was then adjusted to 7.4 using 1N HCl and iN NaOH, sterile filtered (0.22 $\mu$ ) and then stored in a sterile container.

### Trypsin-EDTA solution

Trypsin was purchased as 1x 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).

0.89% Physiological saline

890 mg of sodium chloride was dissolved in 100ml of double autoclaved milliQ water.

### Cell line

Human lung adenocarcinoma-A549 cell line was procured from NCCS Pune, India. The cells were grown in T25 culture flasks containing DMEM medium supplemented with 10% FBS. Detachment of the cells using Trypsin-EDTA solution was employed after confluence..

### Cell proliferation mtt assay

Extract of *Cardiospermum halicacabum* on A549 cell line for the anticancer activity was evaluated using the MTT (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) assay for checking the cytotoxic activity according to the method described. In MTT assay cells were placed in the 96 well plates at the density of 5x10<sup>3</sup>/100 $\mu$ l. The incubation period was 24 hours and the cells were treated with the *cardiospermum halicacabum* extracts in the concentration of 25, 50, 75, 100, 200, 300 $\mu$ g/ml. Wells with serum free medium alone were named as controls. After the incubation period of 24h at 37°C, 10 $\mu$ l of MTT reagent was added to the wells and incubated for 4 hours in the dark. Then, 100 $\mu$ l of sorenson glycine buffer (0.1M glycine, 0.1M NaCl, pH 10.5 with 0.1N NaOH) was added to the wells to solubilise the formazan crystals. The absorbance was measured at 570 nm. The experiment was repeated thrice and each concentration was tested in triplicates. The percentage viability of cells are calculated.

Cell viability (%)=Absorbance of sample/Absorbance of control X 100

### Statistical analysis

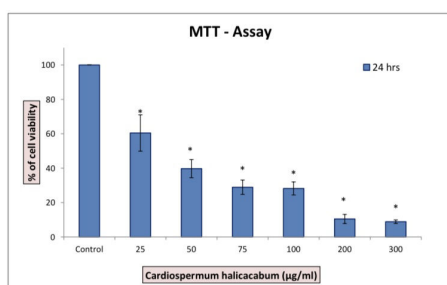
All data obtained were analyzed by Student's-t-test using MS-Excel, represented as mean  $\pm$  SD for six animals in

each group. Statistical analysis utilizing one way ANOVA (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) was performed. For intercomparison including LSD, post-hoc testing was recruited.  $p < 0.05$  was rooted as the statistically significant value.

## RESULTS

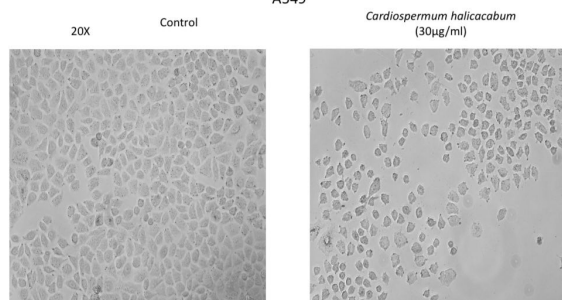
The anticancer activity of *Cardiospermum halicacabum* was determined by MTT assay. From the results of the cell viability assay graph, we observed that the *C. halicacabum* inhibited the cell proliferation upon done and time dependent manner for 24hrs treatment respectively. Finally the *C. halicacabum* inhibit 50% of the cell growth via induce apoptosis at 30  $\mu\text{g/ml}$  (IC50) concentration in lung cancer cells as shown in the Figures 1 and 2.

*In vitro* anticancer activity of *Cardiospermum halicacabum* Against human A549 lung cancer cell line



**Figure 1: The cytotoxic effects of *Cardiospermum halicacabum* on A549 cells. Cells were treated with *cardiospermum halicacabum* (25, 50, 75, 100, 200 and 300  $\mu\text{M}$ ) for 24 h, and cell viability was evaluated by MTT assay. Data are shown as means  $\pm$  SD (n = 3). \* compared with the control-blank group,  $p < 0.001$ .**

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**Figure 2: Assessment of cell morphology of A549 lung cancer cell line treated with *cardiospermum halicacabum* for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope in 20x magnification.**

## DISCUSSION

Lung cancer is the most prevalent cancer in general. Mostly, it is related to cigarette smoke and other factors. Pharmacological plant extracts offer several medicinal qualities, such as antifungal, antioxidant, and antibacterial ones. The purpose of this research was to determine the anticancer efficacy of the plant extract *cardiospermum halicacabum* in a lung cancer cell line. According to the results of the study, at a dose of 30  $\mu\text{g/ml}$ , 50% of the cancer cells are killed. In the study conducted by Nyugen, he said that plant extracts had numerous benefits over chemical components, and the plant extract employed by them was *Adenosma bracteosum* (bonati). Due to the existence of bioactive components such as xanthomicrol and others, it was shown that chlorophyll had substantial efficacy in inhibiting the growth of tumor cells. And he discovered that the plant extract included more cancer-fighting medications. [36,37]. The anticancer efficacy of three plant extracts: *Urtica membranacea*, *Artemisia monosperma*, and *Origanum dayi* was compared in the research. It was discovered that all three plants display anticancer action in a dose-dependent manner. Apoptosis causes cell death. additionally, it was shown that *Urtica membranacea* suppresses breast cancer cell growth directly [38].

Tripathy, reported that the fruit pulp and whole plant extract from *L.acidissima* and *S.cumini* where evaluated hemolytic inhibition assay was also done along with the evaluation of anticancer activity and it was found that the plant extract did not undergo any lysis and it hence it was found that they don't damage the erythrocyte since they lack cardiac glycosides, alkaloids, saponins phlobatannins which are responsible for the damage of erythrocyte. It was also found that they had good anticancer activity against the breast cancer cell line (Tripathy G, et al) [39]. In the study conducted by Shridhar C. Ghagane, he used the leaf sample of *Leea indica* which was subjected to Soxhlet extraction and it tends to increase the polarity of the solvents. It was found that the methanol and ethanolic extract of the leaf was found to have selective anticancer activity in the prostate cell lines and it had no cytotoxic effect on normal embryo fibroblast cells [40]. In the study conducted by Svejda, he used the plant extract of *Trailliaedoxa gracilis*, and the cell line which was involved in the study was human carcinoid KRJ-1 cell line and it was evaluated using cell counting and WST-1 cell proliferation assay. The apoptosis was found using the DAPI staining technique and the results showed that this plant extract has a good inhibiting activity of the carcinoid [41]

Omanike Ogbole in his study analysed the cytotoxic activity of the medicinal plants from Nigeria. He evaluated the extracts using brine shrimp lethality assay and the MTT cytotoxic assay. It was found in his result that *Eleusine indica* showed highest cytotoxic activity on the brine shrimp and the plant extract *Macaranga barteri* Mull. Arg. and *Calliandra portoricensis* Benth showed significant cytotoxic activity against the RD cell line [42]. Anticancer activity of *C.halicacabum* in lung cancer cells by inhibiting cell proliferation by inducing biochemically

and by disrupting cell morphology upon treatment clearly shows the cytotoxic nature of the compound. Therefore, our finding showed the *C.halicacabum* proven as a potent anticancer drug in vitro studies.

### Conclusion

The whole plant extract of *Cardiospermum halicacabum* was hence found to be cytotoxic against the A549 lung cancer cell line and it was found that the plant extract induces apoptosis in a dose and time dependent manner against lung cancer cells. It can be concluded that the plant extract of *Cardiospermum halicacabum* might be an anticancer agent and further studies need to be done for lung cancer treatment.

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