In Vitro Anticancer Activity of Cardiospermum Halicacabum L.Against Human A549 Lung Cancer Cell Line

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Abstract
AIM: The objective of the study was to examine the in vitro anticancer activity of Cardiospermum halicacabum L.Against human A549 lung cancer cell line Methods: Cardiospermum halicacabum was soaked in double distilled water and kept at 37°C for 3 days. The solution prepared was filtered. Fine filtrate was subjected to rota evaporate and 3g of plant extracted sample was obtained. The cytotoxic potency of Cardiospermum halicacabum was carried out by MTT assay against the above mentioned cell line.Furthermore, the morphological changes were analysed using phase contrast microscopy Results: The Cardiosperum halicacabum aqueous extract showed the cytotoxic potency against the MCF-7 cell line which confirmed with greater morphological changes upon 24 hrs treatment. The MTT assay clearly showed that the Cardiosperum halicacabum treatment has significantly reduced the cell viability when the concentration was increased for 24hrs. Conclusion: The present study shows 50% cytotoxic effect at 30 µg/mL of aqueous extract of Cardiosperum halicacabum against human A549 lung cancer cell line.
Keywords: Cardiosperum halicacabum, A549 lung cancer cell line, cytotoxicity

1. Introduction

Cancer is a significant public health concern and is officially listed as the third contributing to death from infectious and cardiovascular diseases(Rajeshkumar et al., 2018).Lung cancer is the third most common cancer and the main cause of cancer-related death in the United States. It is most common in males, and in the U.S., Black males are around 15% more likely to develop it than white males.Smoking is a major risk factor, though not everyone who develops lung cancer has a history of smoking.Lung cancer can be fatal, but effective diagnoses and treatments are improving the outlook.Lung cancer occurs when cells divide in the lungs uncontrollably. This causes tumors to grow(Raghunandhakumar et al., 2013). These can reduce a person's ability to breathe and spread to other parts of the body.Cancer causes changes in cells that are otherwise healthy. The cells grow too quickly, without dying off.Normal cells in the body usually die at a certain stage in their life cycle, thereby preventing a buildup of too many cells. In cancer, however, the cells continue to grow and multiply(Anandakumar et al., 2012). As a result, tumors develop.The two main types of lung cancer are small cell lung cancer and non-small cell lung cancer, depending on how they appear under a microscope. Non-small cell lung cancer is more common than small cell lung cancer.Anyone can develop lung cancer, but cigarette smoking and having exposure to smoke, inhaled chemicals, or other toxins can increase the risk.The main types of lung cancer are non-small cell lung cancer and small cell lung cancer. They differ in the size of cells, as seen under a microscope.Non-small cell lung cancer around 84% of lung cancer cases in the U.S. are non-small cells. There are three subtypes:adenocarcinoma,squamous cell cancer,large cell carcinoma,Small cell lung cancer(Asokkumar et al., 2012).Around 13% of lung cancer cases in the U.S. are small cells. This type tends to grow more quickly than non-small cell lung cancer.People with lung cancer may not have any symptoms until a later stage. If symptoms do appear, they can resemble those of a respiratory infection.(Rajeshkumar et al., 2018; Nandhini, Rajeshkumar and Mythili, 2019)
Radiation, lack of physical activity, obesity and environmental pollutants. These factors can directly

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damage genes or combine with existing genetic faults within cells to cause cancerous mutations(Paramasivam et al., 2015). Approximately 5– 10% of cancers can be traced directly due to inherited genetic defects(Vairavel, Devaraj and Shanmugam, 2020). Chemicals such as tobacco smoke contain over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Alcohol is also a carcinogen.(Gomathi et al., 2020)Excessive free radicals produced during cellular metabolism can attack the deoxyribose DNA backbone and bases which leads to cytotoxicity or mutation and finally it causes cancer(Gomathi et al., 2020; Rajasekaran et al., 2020).

In recent years, there has been considerable emphasis on the identification of plant products with antioxidant property,(Santhoshkumar et al., 2019) as free radicals are considered to play a major role in most of the diseases including cancer. The medicinal value of the chosen plant Cardiospermum halicacabum L bark has been extensively worked out. However, its therapeutic efficacy in antioxidant and antitumor activity has not been evaluated. Cardiospermum halicacabum L. is the member of the family Sapindaceae. The major chemical constituents of C. halicacabum reported to contains (+)-pinitol, β-sitosterol, β-sitosterol-β-D-galactoside, apigenin-7-O-glucuronide, arachidic acid, chrysoeriol-7-O-glucuronide, linoleic acid, lutrolin-7-O-glucudonide, stearic acid. Cardiospermumhalicacabum L. was used to treat various diseases such as skin diseases (rashes, itching, skin irritation, etc.), dandruff, rheumatoid arthritis, gastrointestinal diseases, respiratory tract diseases, urogenital diseases, etc.

There is a necessity for research to search of new compounds with cytotoxic activity, as the treatment of cancer. The available anticancer drug is often unsatisfactory due to the problem, which cause cytotoxicity to the normal cells along with cancer cells. Plants are considered as the valuable sources of bioactive compounds with antioxidant activity, which produce certain substances that have effects on living animal cells. Some extracts rich in apigenin (found in parsley, bell pepper, etc.) causes cancer cells to undergo apoptosis (programmed cell death) in in vitro studies. Our team has extensive knowledge and research experience that has translated into high quality publications (Choudhari and Thenmozhi, 2016; Govindaraju, Jeevanandan and Subramanian, 2017; Ravi et al., 2017; Vikram et al., 2017; Gupta, Ariga and Deogade, 2018; Hannah et al., 2018; Kavarthapu and Thamaraiselvan, 2018; Pandian, Krishnan and Kumar, 2018; Ramamurthy and Mg, 2018; Ashok and Ganapathy, 2019; Ramesh et al., 2019; Sharma et al., 2019; Venu, Raju and Subramani, 2019; Wu et al., 2019; Samuel, Acharya and Rao, 2020).The aim of the present study is to evaluate anticancer activity of Cardiospermum halicacabum L.Against human A549 lung cancer cell line

2. Materials and Methods

Preparation of sample

Cardiosperum halicacabum L powder was commercially obtained from IMPCOPS (Chennai, India). About 150g of sample was soaked in double distilled water and kept at 37°C for 3 days. The solution prepared was filtered with filter paper followed by whatmann paper. Fine filtered samples were concentrated by a rotary vacuum evaporator and the left-over solvent was evaporated to dryness using a hot air oven. 3g of material was obtained and immediately sorted at 4°C.

Chemicals

DMEM (Dulbecco's modified Eagle's medium), 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO were purchased 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. The breast cancer cell line was procured from the National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T25 culture flasks containing DMEM medium supplemented with 10% FBS.

Preparation of Extract

The required quantity of the extra was correctly weighed and dissolved in DMSO with concentration of 1mg/ml as a stock solution. This solution was subsequently diluted to a series of concentrations ranging from 20 to 300 µg/ ml.

3. Mtt Assay

In vitro anticancer activity of Cardiospermum halicacabum were carried out MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay described by Koka(Koka et al., 2018). Further, the viability of A549 cells upon drug treatment was assessed by trypan blue exclusion test. cells were seeded in 96-well plates at the density of 5X103/100µl, after 24hrs cells were treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) of S.virginianum. 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 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:

OD of test

% Of cell viability = ---------- X 100 OD @ 570nm
OD of control

4. Morphological Study

Based on MTT assay we selected the IC50 value of Solanum virginianum for further studies. The characterisation of morphological changes in breast cancer cells before and after treatment with Solanum virginianum were observed under phase contrast microscope.

5. Statistical Analysis

All data obtained were analyzed and 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 $p<0.05$

6. Results

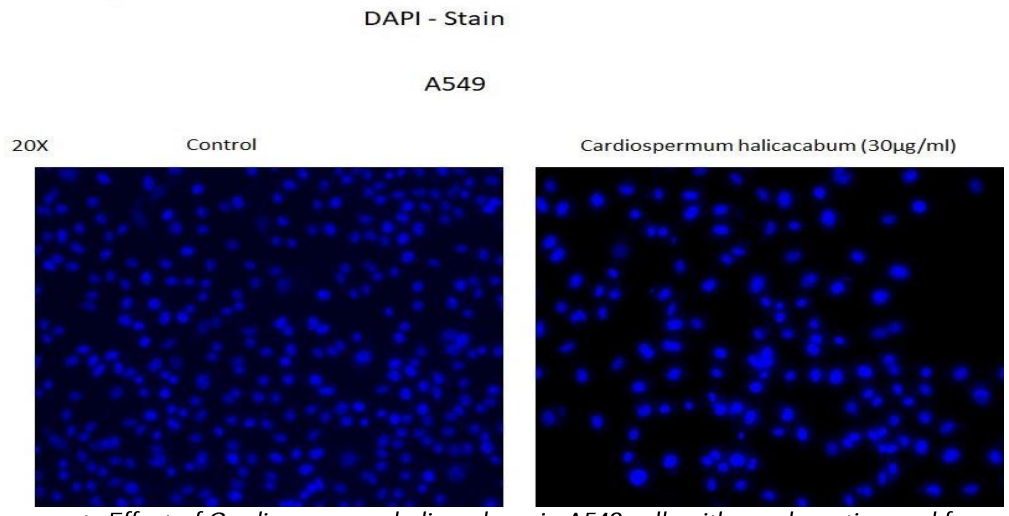

Figure-1: Represents Effect of Cardiospermum halicacabum in A549 cells with condensation and fragmentation of the nuclei as compared with control cells were identified by DAPI with Fluorescence microscopy.

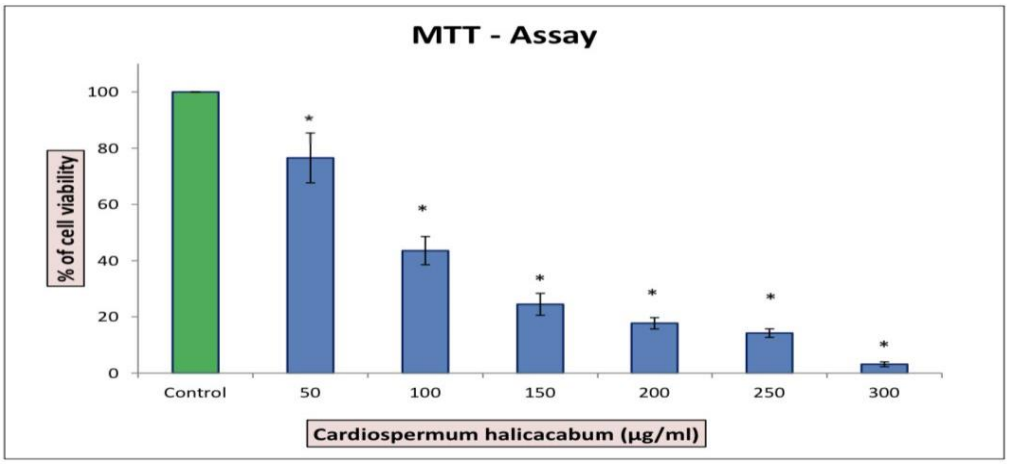

Fig-2: Represents the The cytotoxic effects of Cardiospermum halicacabum on A549 cells was determined by MTT assay. The Cells were treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) for 24hrs. The 50% of inhibition observed in concentration of 30 µg/ml, which has been taken as IC50 value and fixed for further experiments.
*Data are shown as means ± SD (n = 3). * Compared with the control-blank group, $p < 0.001$.*

7. Discussion

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled(Zhang, 2021).Current cancer chemotherapy can damage or kill the rapid dividing healthy cells and causes serious side effects such as neutropesia, anemia, etc(Kang, Zhang and Zhong, 2021). In addition, the cost of chemotherapy drug is high. Natural compounds may reduce these problems. Currently, a few plant products are being used to treat cancer effectively.

Cardiospermum halicacabum L. was well known for its medicinal values (Pinsky, Durham and Strassels, 2021). The preliminary condensation and fragmentation of the nuclei of various extracts of the Cardiospermum halicacabum L. was analyzed. Effect of C. halicacabum in A549 cells with condensation and fragmentation of the nuclei as compared with control cells were identified by DAPI with Fluorescence microscopy. The compounds that inhibit cancer initiation are traditionally termed as blocking agents, this bioactive component present in plants can prevent carcinogenesis by blocking

metabolic activation, increasing detoxification, or providing alternative targets for electrophonic metabolites. They may act by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the level of damage and resulting mutations which contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation(Pandya, Brahmer and Hidalgo, 2016). Bioactivity-guided fractionation of a 90 % methanolic extract of Cardiosperum halicacabum compound displayed anticancer properties against human A549 lung cancer cell line.

This activity of inhibition may be due to the nature of the compounds found in each crude extract and their interaction with metabolic nature of each type of cancer cells(Prakash et al., 2021) or may be due to the effectiveness of some enzymes that act as antioxidants especially in cancer cells.Figure 1 shows effect of Cardiospermum halicacabum in A549 cells with condensation and fragmentation of the nuclei as compared with control cells were identified by DAPI with Fluorescence microscopy and Figure 2 shows the cytotoxic effects of Cardiospermum halicacabum on MCF-7 cells was determined by MTT assay. The Cells were treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 µg) for 24hrs. The 50% of inhibition observed in concentration of 30 µg/ml, which has been taken as IC50 value and fixed for further experiments.

The limitation is that the study does not involve any in vivo study, so its effect is not assessed. This paves way for various future studies such as to view the drug action in in vivo studies and also to know about the side effects of the extract.

8. Conclusion

In this study we have observed that C. halicacabum in A549 cells with condensation and fragmentation of the nuclei as compared with control cells were identified by DAPI with Fluorescence microscopy.

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