

## ANTI CANCER SCREENING OF CARDIOSPERMUM HALICACABUM LEAF EXTRACT ON HUMAN BREAST CANCER CELL LINE

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### ABSTRACT

**BACKGROUND:** To investigate the in vitro antioxidant and anticancer activity of chloroform and ethanol extracts of *Cardiospermum halicacabum* leaves.

**AIM:** The objective of the study was to examine the cytotoxic activity of leaf extract of *Cardiospermum halicacabum* on MCF-7 human breast cancer cell line.

**METHODS:** The cytotoxic activity of *Cardiospermum* was measured by MTT. Briefly, the cells ( $1 \times 10^5$  cells per ml) were seeded in a 96 well microtiter plate (100  $\mu$ l per well) with three individual experiments. Treatment was conducted with different concentrations (20, 40, 60, 80, 100, 200 mg/ml) of cardiospermum extract for 24 hrs. Further the apoptotic effect was evaluated with morphological examination using phase contrast microscopy.

**RESULTS:** The leaf extract of *Cardiospermum* 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 *Cardiospermum* treatment has significantly reduced the cell viability in different doses and time for 24 hrs induction respectively.

**CONCLUSION:** The present study shows 50% ( $IC_{50}$ ) growth inhibition at 3  $\mu$ g/mL of aqueous extract of *Cardiospermum* against MCF-7 human breast cancer cell line.

**Keywords;** *Cardiospermum*; MCF-7; cancer; innovative study

### Introduction

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of deaths after cardiovascular diseases. Cancer is a general term applied to a series of malignant diseases that may affect different parts of the body. (1) These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. Cancer of the female reproductive tract and breast has a high incidence amongst Indian women. The incidence of breast cancer in India is rapidly becoming the number one cancer in females. (2) It is reported that one in twenty-two women in India and one in eight women in America is likely to be victims of breast cancer. Men are also at risk for breast cancer, but the death rate is quite low, at 0.22% or two-tenth of a percent. (3)

Cancer is one of the second leading causes of death worldwide killing more people globally. It is estimated that there are nearly 2.5 million cases in India with nearly 400,000 deaths occurring due to cancer. (4) In India, around 5,55,000 people died of cancer in 2010; according to estimates published in The Lancet Today. Cancer of the female reproductive tract and breast has a high incidence amongst Indian women. (4) The incidence of breast cancer in India is rapidly becoming the number one cancer in females. It is reported that one in twenty-two women in India and one in eight women in America is likely to be victims of breast cancer. (5) Men are also at risk for breast cancer, but the death rate is quite low, at 0.22% or two-tenth of a percent. The plants are the natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals. *Cardiospermum halicacabum* L. is the member of the family Sapindaceae. The major chemical constituents of *C. halicacabum* are reported to contain (+)-pinitol,  $\beta$ -sitosterol,  $\beta$ -sitosterol- $\beta$ -D-galactose. (6)

*Cardiospermum halicacabum* is a plant belonging to the family sapindaceae. It is a deciduous climber growing upto 3 meters. (7) The ground stem carries alternate double triad leaves 3 to 6 cm long, the tiny radiate flowers. Stems are 5 to 10 cm in length. The fruits are tiny green balloon shaped; spherical capsules containing the characteristic seeds with their heart shaped white markings. It is used in the treatment of arthritis, nervous disease, stiffness of the limbs and snake bites. The leaves are applied as poultice in the treatment of rheumatism. (8)The tea made from this is used in the treatment of itchy skin, salted leaves are used as poultice on swellings, and the leaf juice has been used as a treatment for earache. It is also used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite. (9)Hence, the present study is aimed to find the anti-cancer activity with the herbal plant *C. halicacabum* and to analyze the anticancer property against breast cancer cell line.(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)

## MATERIALS AND METHODS

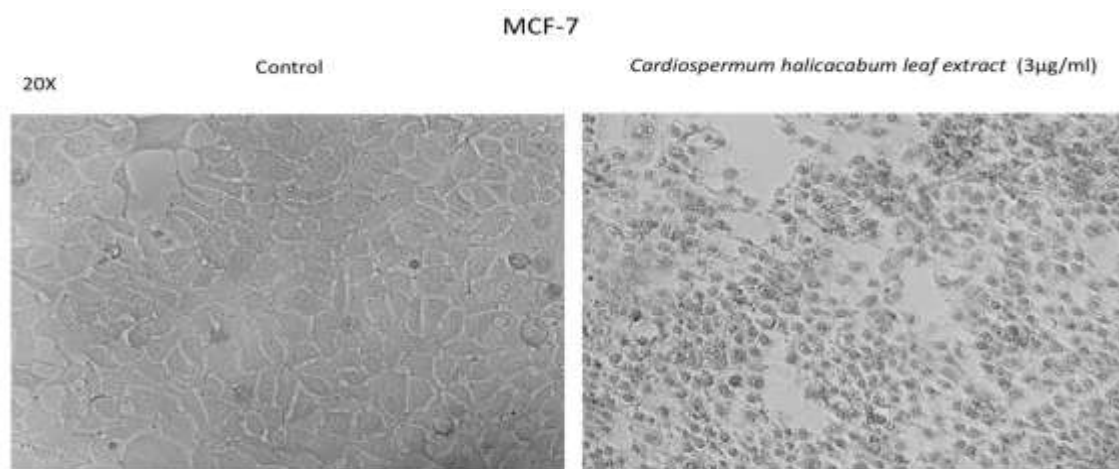
**In vitro cytotoxicity assay** The effect of cardiospermum on cell viability was measured by MTT assay following the method by Briefly, the cells ( $1 \times 10^5$  cells per ml) were seeded in a 96 well microtiter plate (100  $\mu$ l per well) with replications. Treatment was conducted for 24hrs with different concentrations (20, 40, 60,80,100,200mg/ml) of cardiospermum extract . After incubation, 20  $\mu$ l of 3 mg/ml MTT stock solution was added to each well and incubated for 24 hrs at 37 °C. 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:

$$\text{Cell viability (\%)} = \{A570_{\text{od of (sample)}}/A570_{\text{od of (control)}}\} \times 100$$

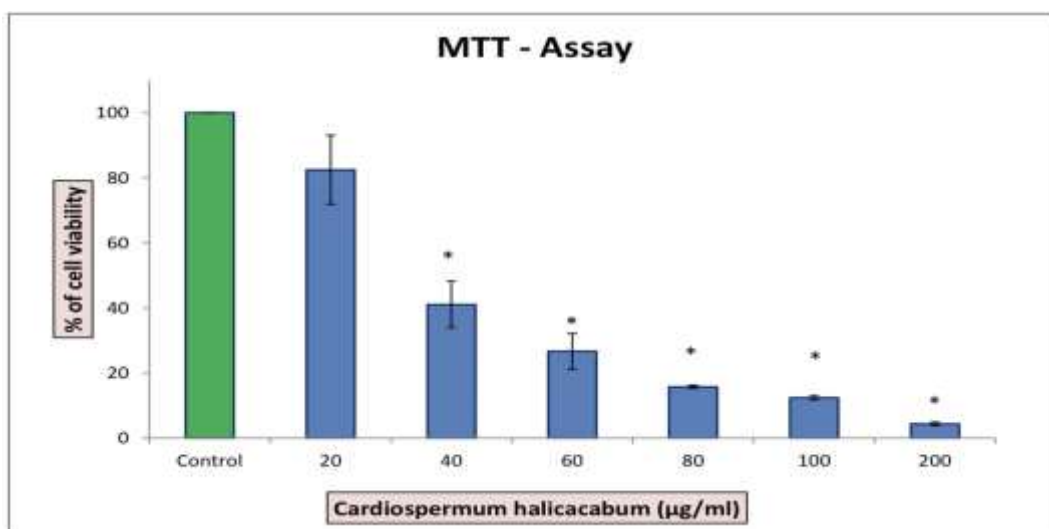
## RESULTS

### Morphology:

Assessment of cell morphology of MDA-MB-231 treated without or with andrographolide. Cells were treated with Lycopene Ag NP (100  $\mu$ M) for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope.



**Figure 1:** Represents the morphological changes in breast cancer cell line upon without (control) and with *Cardiospermum halicacabum* (3  $\mu$ g/mL) treatment for 24hrs. Images were by phase contrast microscope at 20x magnification.



**Figure2:** The cytotoxic effects of Cardiospermumhalicacabum on 3T3 cells. Cells were treated with cardiospermumhalicacabum (20, 40, 60, 80, 100 and 200µM) 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,  $p < 0.001$ .

## DISCUSSION

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 electrophilic metabolites.(31)act by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the extent of injury and resulting mutations which contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation.(32)

From a study conducted it showed the potent cytotoxic activity of n-hexane extract of seeds of *C. halicacabum* Linn against the MCF-7 breast cancer cell line with 50% growth inhibition value (GI50) of 12.8 g/ml but other extracts showed poor activity in other tested cell lines. (33) Other her reported studies indicate the presence of erucic acid, eicosanoic acid, oleic acid, tetradecanoic acid, octanoic acid, and n-hexadecanoic acid as fatty acids in *C. halicacabum*. (34)Here, in this work, we find out the presence of three fatty acids namely palmitic acid, petroselinic acid (omega-12 fatty acid), and paullinic acid (omega 7 fatty acid), and the squalene as a possible responsible compound for the activity. Different secondary metabolites, such as phenolics and flavonoids compounds, and essential fatty acids that are available in many plants are fundamental for humans in the diet and help to lower the risk of heart disease, inflammation, and healing the lipid barriers.(35) Studies of breast and pancreatic cancer in Mediterranean populations have shown that increased dietary intake of oils with squalene such as olive oil is associated with less or no increased risk of cancer, despite a higher proportion of overall lipid intake. (36)In the process of drug discovery, especially from natural and herbal origin, the extraction and fractionation for purification of selected compounds are the main parts of research to find out the responsible phytochemical for targeted activity. (10)In the first stage of research for effect of active compounds on cancer cells, in vitro cytotoxic screening test was usually performed and different values were considered as the indication of cytotoxicity effect, as procedures described by the National Cancer Institute (NCI) and reported by Shoemaker.

From a study conducted it revealed the potential cytotoxic activity of *C. Halicacabum* Linn seeds. against MCF-7 breast cancer cell line with 50% growth inhibition value (GI50) of 12.8 µg/ml and cytotoxicity effect of fractions of seed against MCF-7 breast carcinoma with 50% growth inhibition value (GI50) of <10 µg/ml. The GC-MS analysis suggested the presence of active components that led to this result. (37)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.

## CONCLUSION

The present study shows that the Cardiospermumhalicacabum has potent anticancer activity and at 3µg/mL concentration inhibits 50% of the cell proliferation at 24hrs respectively(38-47). Further investigation at pre-clinical and clinical levels for establishing it as a potential agent for cancer therapy.

## ACKNOWLEDGEMENT

The team extends our sincere gratitude to the Saveetha Dental College and hospitals for their constant support and successful completion of this work.

#### CONFLICT OF INTEREST

Nil k

#### SOURCE OF FUNDING

The present study was supported by the following agencies

- Saveetha Dental College
- SIMATS, Saveetha University
- Anbu Offset AchagamPvt Ltd

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