Research Article

Cytotoxic Activity of *Cardiospermum halicacabum L.* against Oral Cancer Cell Lines.-SCC25

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ABSTRACT

Aim: To evaluate the Cytotoxic activity of *Cardiospermum halicacabum L.* against oral cancer cell lines (SCC25). **Method:** Oral cancer cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 × 10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (25, 50, 75, 100 and 125 µg/ml) for 24 hours. After the incubation, medium was discarded and 100 µl fresh medium was added with 10 µl of MTT (5mg/ml). After 4 hrs, the medium was discarded and 100 µl of DMSO was added to dissolve the Formosan crystals. Then, the absorbance was read at 570 nm in a microtitre plate reader. Cyclophosphamide was used as a positive control for the Cancer cell lines. Medium along with cells (untreated) serves as a control. **Results:** Our present study showed the cytotoxicity levels of *Cardiospermum halicacabum L.* in conjunction with a positive control Cyclophosphamide at different concentration (25 µg/mL,50µg/mL,75 µg/mL, 100 µg/mL, 125 µg/mL). Cyclophosphamide is chosen as positive control as it's known for its cytotoxicity ability in cancer cell lines. The cytoxicity level seems to increase with increase in concentration of the compound. While comparing test compound with the positive control the cytotoxicity levels of the test compound were as less efficient as the cyclophosphamide but within the compound concentrations had good cytotoxicity capacity. **Conclusion:** Hence furthermore investigations with higher concentrations and using this product with other compounds as adjuvant can give a better result.

Key words: Cytotoxicity, Cancer cell lines, *Cardiospermum halicacabum L*, cyclophospahmide, Oral cancer.

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INTRODUCTION

Cancer is the term which indicates the uncontrollable programmed growth of the abnormal cell in the body. It can occur in any part of the body which disturbs the normal mechanism of a working cell. There are different types of cancers which are the leading cause of death in worldwide [1] Cancer is considered one of the most important health issues in developed and developing countries and each year, the number of patients diagnosed with cancer is increasing globally.[2] Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year.[3] More than 30% of cancers are due to behavioral and environmental changes. Tobacco is the biggest cause of cancer, which is responsible for up to 1.5 million cancer deaths a year. Chemicals such as tobacco smoke contain over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Alcohol is also a carcinogen. [4] 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. Free radicals also a factor which causing all types of cancer. [5]

There are many treatments for cancer such as surgery, chemotherapy and radiation therapy. Existing chemotherapy and treatment leads to different painful side effects. Many herbal medicines have been mentioned for the treatments of different diseases including cancer. It has proven that plant compounds present in

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the human diet with many active constituents can help for prevention and cure of disease. Many chemotherapeutic agents singly or in combination demonstrate the activity against cancer and among the chemotherapeutic agents, plant-based medicine is used for treating the patients with cancer like oral cancer. [6]

Cardiospermum halicacabum Linn. Belongs to *Sapindaceae* family has been known as medicinal plant that was used by various traditional system of medicine, which possesses therapeutic effect and has nutritional values. It was used for the treatment of different ailments in various medicinal systems such as Ayurveda, the Indian System of Medicine, Unani System of Medicine and Traditional Chinese Medicine. [7] In Ayurveda, it has been used for the treatments of limbs stiffness, snake-bites and chronic bronchitis. In Chinese medicine, it has been used for the treatment of rheumatism, nervous diseases, lumbago and dropsy. [8] In Unani medicine, seeds were mentioned as tonic and used for the treatment of cancer.[9]

MATERIALS AND METHODS

Plant material

The plant extract is provided as gift sample from Life care phyto labs Pvt Ltd, Chennai, Tamilnadu, India for conducting the study.

Chemicals

Phenol free Dulbecco's modified Eagle medium (DMEM), MTT, Dimethyl sulphoxide (DMSO), phosphate buffer saline (PBS) and antibiotic-cyclophosphamide were purchased from Sigma-Aldrich. Fetal bovine serum was purchased from GIBCO/BRL Invitrogen.

Cell culture

Oral squamous carcinoma cell lines SCC25 were obtained from the NCCS, Pune with Passage no 16. Cells were cultured in phenol red-free Dulbecco's modified Eagle medium (DMEM) supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal bovine serum at 37°C with 5% CO_2. Cells were washed with DMEM medium and detached with 0.25% trypsin-EDTA. The cells were re-suspended in DMEM medium at a density of 2×10^6cells/ml.

MTT assay for Cytotoxicity

The MTT assay [10] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formaz an product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO_2.

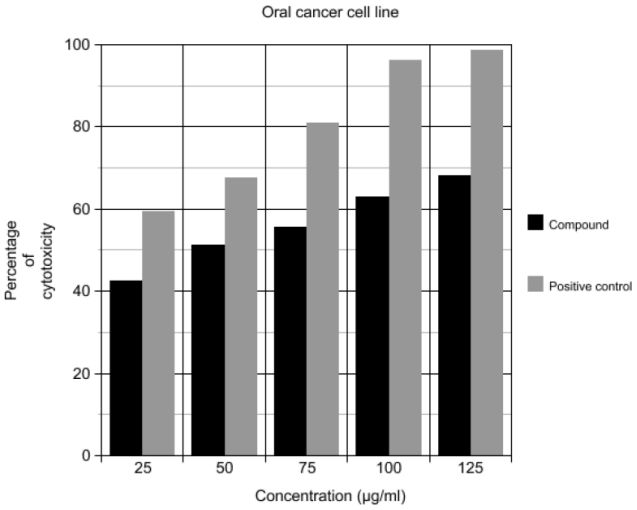

Figure 1: Cytotoxicity analysis.

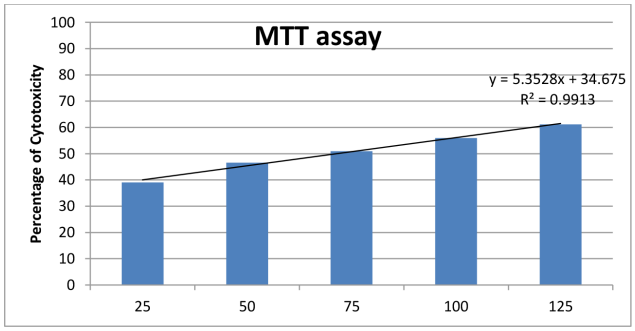

Figure 2: Graph showing Cytotoxicity.

SCC25 cell lines were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2×10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded, and cells were incubated with different concentrations of the samples (25, 50, 75, 100 and 125 µg/ml) for 24 hrs. After the incubation, medium was discarded and 100 µl fresh medium was added with 10µl of MTT (5 mg/ml). After 4 hrs, the medium was discarded and 100 µl of DMSO was added to dissolve the formaz as a crystal. Then, the absorbance was read at 570 nm in a microstate plate reader. Cyclophosphamide was used as a positive control for the Cancer cell lines. Medium along with cells (untreated) serves as a control.

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability \%}$$

RESULTS

Our present study shows the cytotoxicity levels of *Cardiospermum halicacabum L*.in comparison with a positive control Cyclophosphamide at different concentration (25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, 25 µg/mL) Showed in Table 1, 2 and 3. Cyclophosphamide is chosen as positive control as it's known for its cytotoxicity ability in cancer cell lines. The cytoxicity level seems to increase with increase in concentration of the compound as depicted in Figure 1 and 2. While comparing test compound with the positive control the cytotoxicity levels of the test compound were as less efficient as the cyclophosphamide but within the compound concentrations had good cytotoxicity capacity. Hence furthermore investigations with higher concentrations and using this product with other compounds as adjuvant can give a better results.

DISCUSSION

Cancer is a series of molecular events that 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. [11] Current cancer chemotherapy can damage or kill the rapid dividing healthy cells and causes serious side effects such as neutropenia, anemia, etc. 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. A study was made to develop an anticancer drug from a common plant source. We found that plant belonging to the family Sapindaceae is well documented for their anticancer potential. [12] *Cardiospermum halicacabum L.* was well known for its medicinal values. The preliminary phytochemical screening of various extracts of the *Cardiospermum halicacabum L.* was analyzed. Chloroform extract contains alkaloids, coumarin, flavones, quinones, saponins, steroids and tannins. Ethanol extract showed the presence of alkaloids, coumarin, flavones, quinones, saponin, terpenoids, steroids and sugar. Strawberry methanolic extract (SBE) was prepared and cytotoxic activity of different concentration of SBE on KB cell lines was determined by (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and neutral red dye incorporation test. Strawberry extract exhibits cytotoxic activity over the oral cancer cell lines. On administration of about 100 µg/ml of strawberry extract, about 50% of cell viability could be observed and assessed from the cell lines. Strawberries have a cytotoxic effect on oral cancer cell line due to the presence of anticancer constituents in the berries. These berries can be used as a natural medicine for cancer sufferers.[13]

Several studies have revealed that certain naturally occurring medicinal plants inhibit the growth of various cancers. A study was conducted to evaluate cytotoxicity and apoptotic induction potential of *Myristica fragrans* Houtt mace extract. The cytotoxic activity of the *Myristica fragrans* Houtt mace acetone extract was assayed by MTT assay. The apoptotic induction potential was also studied by the analysis of Bcl-2 protein and gene expression in mace extract incubated KB cell lines using western blotting technique and real-time polymerase chain reaction. The mace extract exhibited cytotoxicity and anticancer effect against KB cell lines and it also suppressed the growth of cancer cells, therefore growth inhibitory effect was noted in extract treated cell lines. The mace extract shows the cytotoxic activity and induced the apoptosis through the modulation of its target genes Bcl-2 in the KB cell lines, suggesting the potential of mace as a candidate for oral cancer chemoprevention.[14]

Table 1: Average OD values of compound and PC.

	Average OD		
Concentration (µg/mL)	Compound	Positive Control	Control
25	0.4977	0.3519	0.8659
50	0.4227	0.2808	0.8659
75	0.3845	0.1641	0.8659
100	0.3215	0.0336	0.8659
125	0.2765	0.0121	0.8659

Table 2: Cytotoxicity of Sample and Positive control in oral cancer cell line.

	Oral cancer cell line	
Concentration (µg/ml)	Compound	Positive Control
25	42.52	59.36
50	51.18	67.57
75	55.59	81.04
100	62.87	96.12
125	68.06	98.61

Table 3: IC50 value of compound in Oral cancer cells (in µg/mL).

	Compound
IC_{50} (µg/mL)	71.58

CONCLUSION

The present study revealed that the *Cardiospermum halicacabum* exerts anti-cancer effects against oral squamous carcinoma cell lines. Overall, these findings provide evidence that this extract can act as a chemo protective agent.

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CONFLICT OF INTEREST

The authors declare no conflict od interest.

ABBREVIATIONS

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **SCC25:** Oral squamous carcinoma cell line; **NCCS:** National Centre for Cell Science.

REFERENCES

1. Gennari C, Castoldi D, Sharon O. Natural products with taxol-like antitumor activity: Synthetic approaches to eleutherobin and dictyostatin. Pure Appl Chem. 2007;79(2):173-80.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7-30.
3. Kinzler, Kenneth Vogelstein and Bert. The genetic basis of human cancer. J New York: McGraw-Hill, Medical Pub. 2002;005-9.
4. Kuper H, Boffetta P, Adami HO. Tobacco use and cancer causation: Association by tumour type. J of Internal Medicine. 2002;252(3):206-24.
5. Ellestad GA. Structural and conformational features relevant to the anti-tumor activity of caliche amiciny. J Chirality. 2011;23(8):660-71
6. Khlifi S, ElHachimi Y, Khalil Y, Khalil A, ES-Safi N, *et al. In vitro* antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract. Indian J Pharmacol. 2006;38(4):276-80.
7. Mohaddesi B, Dudhrejiya A, Sheth NR. Anticancer screening of various seed extract of *Cardiospermum halicacabum* on human colorectal, skin and breast cancer cell lines. Arch Breast Cancer. 2015;2:91-5.
8. Venkateshbabu KC, Krishnakumari S. *Cardiospermum halicacabum* suppresses the production of TNF-alpha and nitric oxide by human peripheral blood mononuclear cells. Afr J Biomed Res. 2006;9(2):95-9.
9. Ahmad J, Malik AA. Botanicals used in Unani system of medicine for cancer chemoprevention and cancer therapy. Herbal Medicine – A Cancer Chemopreventive and Therapeutic Perspective. New Delhi: Jaypee Brothers Medical Publishers. 2010;73-64.
10. Tim M. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2): 55-63.
11. Nagendra P, Shahira E, Laila I. Antioxidant and anticancer activities of high pressure-assisted extract of longan (*Dimocarpus longan* Lour.) fruit pericarp. J Innovative Food Sci and Emerging Technologies. 2009;10(4):413-9.
12. Raju SK, Balasubramanian R, Perumal P. *In vitro* and *in vivo* anticancer activity of *Indigo fera cassioides* Rottl. Ex. DC. Asian Pacific J of Tropical Medicine. 2011;4(5):379-85
13. Ramya G, Vishnu PV, Gayathri R. Cytotoxicity of Strawberry Extract on Oral Cancer Cell Line. Asian J Pharm Clin Res. 2018;11(9):353-5.
14. Gayathri R, Anuradha V, Vishnu PV, Mallika J. Cytotoxic and apoptotic potential of *Myristica fragrans* Houtt. (mace) extract on human oral epidermal carcinoma KB cell lines, Braz. J Pharm Sci. 2018;54(3):e18028.

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