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In vitro cytotoxic activity of methanolic extract of *Cardiospermum canescens* Wall. (Sapindaceae)

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The present study was designed to evaluate the *in vitro* cytotoxicity activity of methanolic extract of *Cardiospermum canescens*. In this study the extract was tested using human cancer cell lines, Human ductal breast cancer cell line and Colon cancer cell line for their effects on cell viability, growth inhibition and cell morphology. Cell viability and inhibition were determined by MTT [(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] assay by using DAPI (4- 6-diamidino- 2- phenylindole) staining technique. The significant decrease in cell viability was observed for 100µg/ml in human ductal breast cancer cell line and 150µg/ml in colon cancer cell line. The IC_{50} value is 31.25µg/ml and 250µg/ml in ductal breast and colon cancer cell lines respectively. The results indicated that methanolic extract of *Cardiospermum canescens* has a potential cytotoxicity activity on Human ductal breast cancer cell line than the colon cancer cell lines.

Keywords: *Cardiospermum canescens,* MTT-assay, DAPI staining, Human ductal breast cancer and colon cancer cell lines.

Introduction

Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology (Xu *et al.,* 2009). There are various medicinal plants reported to have anti-cancer activity because the treatment has low cost and low side effects.

Cardiospermum canescens Wall, known as the Balloon plant or Love in a puff, which is a climbing plant widely distributed in tropical and subtropical Africa and Asia. The genus *Cardiospermum* (Family Sapindaceae) represents more than 30 recognized species throughout the world (Quattrocchi, 1999). In rural south India, it has been harvested and sold in urban and local market as green vegetable providing a source of revenue for low-income families. *Cardiospermum* is an active ingredient in creams and lotions for dermatitis, eczema and

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psoriasis. The whole plant is applied to reduce swellings and hardened tumors. There is a claim that roots are used by some local tribes to treat rheumatoid arthritis in Asian and African communities (Kirtikar and Basu, 1984).

Materials and Methods

Preparation of Methanolic extract

10 g of *C. canescens* was weighed and soaked in 50 ml of 98 % methanol for 5 days. The extract was then filtered using Whatman filter paper No.1 and the filtrate was concentrated by vacuum evaporator. Then the obtained residue was used for further studies.

Cytotoxicity determination by MTT assay

T-47 and HT 29 cancer cells were sub cultured in DMEM media supplemented with 2mM L-glutamine adjusted with 1.5g/L Sodium bicarbonate and 90% fetal calf serum incubated at 37^{o}C in 5% CO_2 incubator. Serial dilution of *C. canescense* filtrate (1.953-1000µl) was added to the T-47 and HT 29 cancer cells (6 µl), seeded in 96-well microtiter and incubated at 37^{o}C for 24 hours. At the end of the treatment, 20 µl of MTT [(3, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well and the microtiter plates were incubated for 4 hrs at 37^{o}C. Finally, acidic isopropanal (100µl) was added to each well, after which optical absorbance was read at 595nm on multi well spectrophotometer plate reader (Arung *et al.,* 2006).

The percentage viability was calculated as follows:

Cell Viability = Optical density of samples /Optical density of control X 100.

Cell Morphological Studies

DAPI staining was performed as described by Sandra *et al.*, (2002) for the determination of morphological changes of cells were seeded on glass slide and treated with plant extracts for 24 hours. Untreated and treated cells were rinsed with phosphate buffered saline, fixed with ice- cold 10% trichloroacetic acid and further washed with cold 70%, 80% and 90% of ethanol. The cells were stained with 1µg/ml 4- 6-diamidino- 2- phenylindole (DAPI) for 3 minutes cover slipped with 90% glycerol and observed under floroscence microscope.

Statistical analysis

The IC_{50} (Inhibitory concentration) is the concentration of the toxic compound that reduces the biological activity by 50%. The IC_{50} value was obtained from MTT assay and calculated using Microsoft excel software. The values were expressed in geometric mean. Differences were considered to be a statically significant when $P < 0.05$ and $P < 0.01$.

Results and Discussion

The *In vitro* cytotoxicity of methanolic extract of *C. canescens* was studied using various concentrations by MTT assay method. Human ductal breast cancer cell line (T-47) and Human Colon cancer cell line (HT-29) were used for this study. The cell viability, growth inhibition and morphological changes were compared with untreated cells. Decrease in cell viability and increase in growth inhibition was observed on the two cell lines in dose dependent manner. The significant decrease in cell viability ($P < 0.01$) was observed for 100µg/ml in human ductal breast cancer cell line and the Inhibitory Concentration (IC_{50}) value is 31.25µg/ml. The methanolic extract of *C. canescens* showed the good inhibition in the concentration of 150µg/ml in colon cancer cell line and the IC_{50} value is 250µg/ml. Table 1 and 2 shows the percentage

viability and percentage inhibition of the treated cells with different doses of methanolic extract of *C. canescens*. *C. Canescens* methanolic extract significantly reduce the human ductal breast cancer cells growth.

DAPI staining was conducted to investigate methanolic extract of *C. canescens* induced changes in cell structures. Cells were incubated with methanolic extract of *C. canescens*, and morphological alterations were confirmed via floroscence microscope. As shown in Figure 1 after 24 h of incubation with various concentrations of plant extract, many of the cells showed cytoplasmic shrinkage and loss of normal nuclear architecture, became detached and found floating in the medium. As a result, the number of cytotoxic cells increased with the effect of various concentrations of plant extract was recorded.

Table 1: *In vitro* cytotoxic activity of *Cardiospermum canescens* against Human ductal breast cancer cell line (T-47)

S. No	Concentration (µg/ml)	Dilutions	Human ductal breast cancer cell line
1	1000	Neat	28.45 ± 1.19
2	500	1:1	29.90 ±1.55
3	250	1:2	37.78 ±1.33
4	125	1:4	48.61 ±1.26
5	62.5	1:8	49.08 ±0.78
6	31.25	1:16	51.67 ±1.55
7	15.625	1:32	54.89 ±0.56
8	7.8125	1:64	60.45 ±1.84
9	3.906	1:128	73.66 ±1.31
10	1.953	1:256	88.09 ±1.09
11	Cell control	-	100
IC_{50}			31.25µg/ml

The pausing of literature indicates that this family Sapindaceae has potent anticancer activity. The Bio-assays of extracts from *Sapindus trifoliatus* showed that a fraction (fraction 3) from an ethanolic extract had an antiproliferative effect on SKBR3 and MDA-MB435 human breast cancer cells. The Effective Concentration (EC_{50}) value of *S. trifoliatus*, fraction 3 was 56.07 and 30.61µg/ml for SKBR3 and MDA-MB435, respectively (Pradhan *et al.,* 2010). Cytotoxic potential of *Aesculus indica* crude leaf extract and its fractions was investigated against MCF-7 cell line by MTT assay. Cell viability was inhibited by *A. indica* crude extract in a dose dependent manner ranging from 34.2% at 10µg/ml to 94% at 500µg/ml. Activity was found in an ascending order from hexane showing 29.8% inhibition to aqueous fraction indicating maximum inhibition, 60% (Bibi et al., 2012). The extracts of *Schleichera oleosa* was initially assessed for their *in vitro* cytotoxicity potential in the sulforhodamine B dye assay against cell lines, such as 502713 (colon), SW-620 (colon), HCT-15 (colon), A-549 (lung), HEP-2 (liver), SK-NS-H (central nervous system), and IMR-32 (neuroblastoma). It was observed that the water extract was effective against all the three Colon cancer cell lines, while only methanol and water extracts were effective against A-549 (lung) and HEP-2 (liver), respectively(Thind *et al.,* 2010).

Conclusion

In this study, the effective concentrations of methanolic extract of *C. canescens* showed significant inhibition of cancer cells. Therefore, purification for *C. canescens* extract in future studies is recommended. Also, in regards to the significant results of this study, further investigations such as evaluation of *in vivo* anticancer activity of *C. canescens* is recommended and may lead to finding new effective natural anti-tumor compound(s).

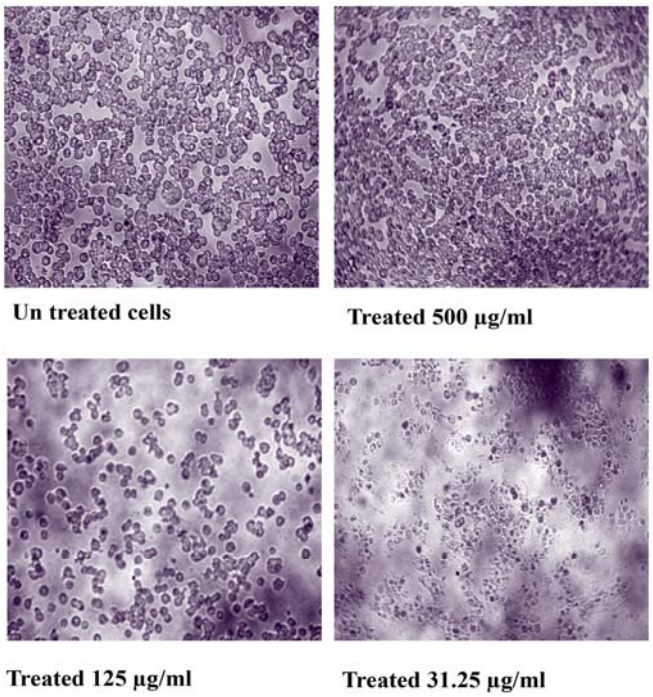

Fig 1: *In vitro* cytotoxic effect of *Cardiospermum canescens* on human ductal breast cancer cell line (T-47) using DAPI staining

Table 2: *In vitro* cytotoxic activity of *Cardiospermum canescens* against Human colon cancer cell line (HT-29)

S.no	Concentration (µg/ml)	Dilutions	Human colon cancer cell line HT 24
1	1000	Neat	32.56 ± 1.29
2	500	1:1	43.77 ±1.80
3	250	1:2	56.67 ±0.71
4	125	1:4	58.09 ±0.66
5	62.5	1:8	64.32 ±0.33
6	31.25	1:16	68.55 ±1.29
7	15.625	1:32	73.82 ±1.59
8	7.8125	1:64	89.09 ±1.92
9	3.906	1:128	94.56 ± 1.47
10	1.953	1:256	98.78 ±1.13
11	Cell control	-	100
IC_{50}			250µg/ml

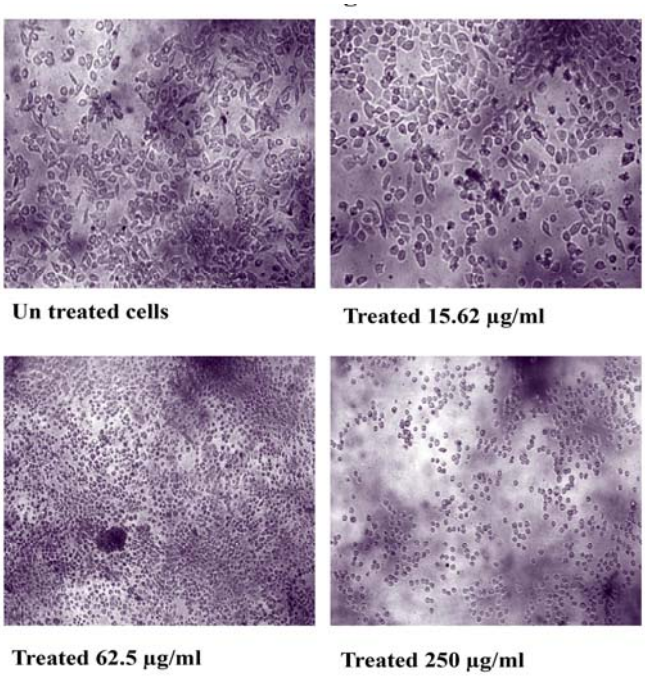

Fig 2: *In vitro* cytotoxic effect of *Cardiospermum canescens* on human Colon cancer cell line (HT 29) using DAPI staining

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