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Effect of ethanolic extract of *Reissantia indica* on the human breast cancer cell line (MCF-7)

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ABSTRACT

Reissantia indica belongs to the family Celastraceae, is a native shrub to Maharashtra and Bengal, India. Its distinct plant parts are said to possess different activities, natives of Maharashtra and Bengal are using the plant parts for various purposes. In this experiment, the effect of ethanolic extract to destroy the cancer cells was studied. Study was carried on with human breast cancer cell line (MCF-7). Preparation of cell line was done using the reagents under standard protocol. Effect of plant extract on the cancer cells were recorded at two different timings (24 & 48 Hrs) respectively. Various doses of plant extract and standard were taken and introduced into cell line. Using 96 well plates the cells were seeded and studied for the viability. From the tabulated records, it clearly shows us the dose dependent response for the inhibition of cells. The maximum concentration of the plant extract 100µg/ml was found to be effective.

Keywords: MCF-7, Cancer, Breast cancer, Cell line, Viability.

INTRODUCTION

Reissantia indica [1]; Synonym: Hippocratea indica willd, Pristimera indica. It has its habitat as North-eastern India. Specifically the root bark is used for the treatment of respiratory troubles and stems are used as febrifuge. Likewise, powdered leaves and roots for application on wounds and sores. The chemical compound that is present in root is dulcitol and an antibiotic principle Pristimerin (0.1%) which is considered to possess activity against Gram positive Cocci. It is believed that pristimerin is also works against the strains of mycobacterium which causes tuberculosis. It is useful as an adjunct to common antibiotic therapy of respiratory inflammations. Cancer is a dreadful disease that features the uncontrollable cellular growth, metastasis and local tissue invasion. Treatment for cancer is always been costly and express several side effects with respect to treatment like radiation or chemotherapy.

In this experiment, we have chosen human breast cancer cell line MCF-7 for the investigation of anticancer property by performing cytotoxicity assay. After the authentication of the plant, the extraction procedures and MTT assay [2] were carried out. Completing the series of procedures, the solution was subjected to absorbance study and the reagent absorbance and cell absorbance was subtracted for the absorbance exhibited by the viable cells at 650 & 570nm respectively.

MATERIALS AND METHODS

Collection and processing of the plant

The aerial parts of the plant *Reissantia indica* was collected from sengotai, Tirunelveli, Tamilnadu, India in the month of November, 2016. Plant material was identified and authenticated by Mr. V. Chelladurai, Retired research officer botany, C.C.R.A.S. Govt of India, Tirunelveli. The collected plant was free from diseases and also free from contamination of other plants. The collected plant was air dried for few days and pulverized.

Preparation of extract

Two kilograms of the powdered was cold-macerated with 70 % v/v of ethanol for 48hrs and 72hrs respectively. The hydroethanolic extract was then concentrated to a syrupy mass under reduced pressure in soxhlet apparatus, air-dried and preserved in a silica desiccator. The phytochemical analysis of the extract was also performed.

Anti-cancer assay [2]

Cancer assay is usually performed with relation to MTT reagent. The uncontrollable growth and differentiation of cells is cancer and preventing the growth of cells will automatically lead to suppression of cancer cells.

Principle

Basic principle underlying is the, viable cells take up the MTT reagent and will exhibit colors in accordance with the viability. MTT which is chemically known as (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide). In this assay, the active cells are expected to turn the yellow tetrozolium to formazan, a purple color complex. The resulting intracellular formazan complex is solubilized using DMSO and quantified spectrophotometrically at 570nm.

Reagents required

- 10% FBS
- Penicillin (100U/ml)
- Streptomycin
- PBS (Phosphate buffer saline)
- DMSO (Dimethyl sulfoxide)
- MTT

Procedure

MCF-7 cell line was maintained in the growth medium RPMI – 1640 (Roswell park memorial Institute). It contains 10% FBS, penicillin and streptomycin. Now the cultured cells are seeded in the 96-well plates. Cells were incubated at 37ºC for 36 hours. After incubation the cells were treated with various concentration of ethanolic extract (3.12, 6.25, 12.5, 25, 50, 100µg/ml respectively). Then phosphate buffered saline along with the MTT earlier dissolved, was added to well and was incubated at 37ºC for 4 hours. Optical density (OD) of the plates was read in the multi-well plate reader at a test wavelength of 570nm and a reference wavelength of 650nm. As per the values obtained, they were tabulated.

Formula

Percentage Cell Inhibition =

[100- Absorbance (sample)/Absorbance (control)] x100.

RESULTS AND DISCUSSION

The phytochemical analysis of the ethanolic extract of aerial parts *Reissantia indica* reveals that the plant has components [3] such as alkaloids, terpenoids, steroids, flavanoids, phenolic compounds, saponins, tannins and glycosides. It is observed that the ethanolic extract[4] has high anti-cancer activity. Precedent studies [4, 5] with plant species regarding any activity has promising results with veneration to ethanolic extracts when compared to other solvents.

Table 1: Percentage viability

Concentration (µg/ml)	OD (570 - 650 nm)	% Cytotoxicity	% Viability
3.125	0.788	7.973525	92.02647
6.25	0.766	10.46136	89.53864
12.5	0.633	26.07358	73.92642
25	0.552	35.47985	64.52015
50	0.414	51.57485	48.42515
100	0.219	74.37415	25.62585
DMSO 1	0.470	49.09247	54.90753

The greatest restraint of the MCF-7 cells at the 100µg/ml focus was observed to be 74.37% inhiition. As the analysis was completed with various measurement and in an expanding focus, the rate lethality was additionally expanding as for fixation. With respect to the optical density of the light passed and also considering the formazan formation, the results were recorded. The %Cytotoxicity was calculated using the formula. Percentage viability was calculated from the value obtained after using formula, which indicates the percentage of live cells.

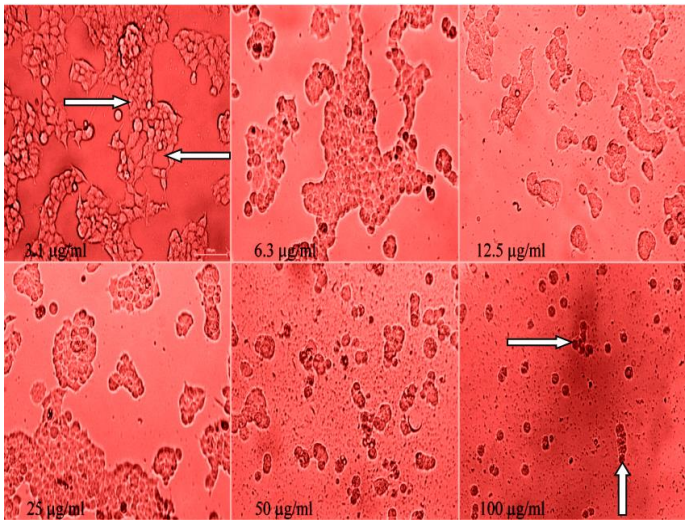
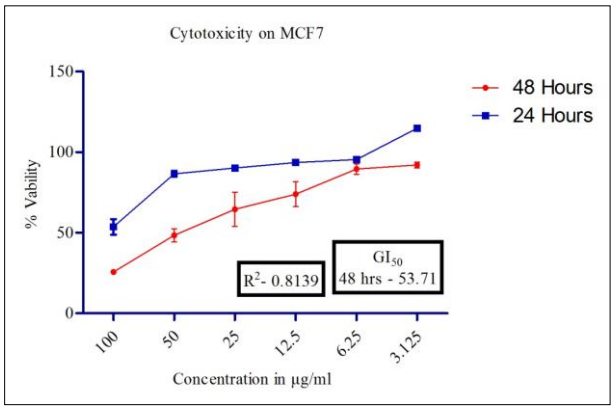


Figure 1: Cells after the administration of various concentrations of plant extract

The Fig 1 clearly shows the inhibition of growth of cancer cells which decreases with respect to the dose administered. At the final dose, there was only minimal amount of cells survived. In the graph [5], within the time span of 24 hours we are able to the growth cessation for cancer cells. The growth inhibition of the extract was found to be 53.71µg/ml at 48 hours. It is believed that the cytotoxicity activity will be due to the presence of flavanoids. Obtaining results nearing 74% as inhibition value is really of great importance, and it is proved to be a anticancer agent.



Graph 1: %Viability Vs Concentration (µg/ml)

There was a measurement subordinate reaction in cytotoxicity thinks about for every one of the fixations tried. In-vitro presentation of MCF-7cells with different convergences of *Reissantia indica* fundamentally stifled the development of cells in a measurements subordinate way.

CONCLUSSION

Reissantia indica improves overall symptom associated with cancer without any toxic side effects. It may be a potential chemotherapeutic agent based on its ability to induce apoptosis in cancer cells. Therefore it can be recommended for the development of herbal formulation for cancer treatment.

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