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Periodic Research

In-Vitro Anti-Cancer Activitymethanolic Extract of Roots of Puerariatuberosa

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Abstract

The HeLa and MCF-7 cells were treated with Methanolic extract of Pueraria tuberose and effects on the cytotoxic nature of extract of Puerariatuberosa. Were determined using Trypan blue and Hoechst tests. The MTT assay results were used to evaluate the anti-cancerous activity of the extract.The effects of plant Roots extract on cancer cells was studied. The IC50 (1.35µg/µl to 1.2µg/µl.) values was found for the extract.The nature of cytotoxic activity is found to be apoptotic rather than necrosis by Hoechst test. The anti cancer effects of methanol extract of Pueraria tuberose Roots may be related to their content of flavonoids. This study validates the traditional use of the plant in management of Cancer.

Keywords: Puerariatuberosa, Anti cancer activity.

Introduction

Cancer[4] is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. It is a generic term for a group of more than 100 diseases that can affect any part of the body. Various plant parts are extracted for the treatment of cancers. Although there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Accordingly several new strategies are being developed to control and treat cancer. One such approach could be a combination of an effective photochemical with chemotherapeutic agents, which when combined would enhance efficacy while reducing toxicity to normal tissues. Puerariatuberosais a prickly, glabrous, branching herb with yellow juice and showy yellow flowers native to Tropical America. In India it is introduced and naturalized and occur as wasteland weed in almost every part of India. It has been reported to exhibit Diuretic, Purgative and Deatroys worms. It cures Leprosy, Skin diseases, Inflammations and Bilious fevers. Alkaloids and flavonoids are reported to be the major phytoconstituents. The aim of the present study was to evaluate the effect of the methanolic extract of PuerariatuberosaRootsonHeLa and MCF-7 cell lines.

Materials and Method

Plant Material

The Roots were collected in the month of March 2007 from SFRI Jabalpur. The species for the proposed study was identified and authenticated as Puerariatuberosa. belonging to Family legumase by Late Dr. J. L. Shrivastav, SFRI Jabalpur (M.P.)

Extraction

The powdered material of Roots (30g) was extracted with petroleum ether by using Soxhletapparatus (continuous hot percolation at 75^0C) to remove fatty substances. The extract is dried under reduced pressure by using rotary evaporator. The residue was then stored in a desiccator. The powder material (marc) remaining is further extracted with methanol. The HPLC study was identified and authenticated as roots of Pueraria tuberose present major isoflavnoids. (fig1)

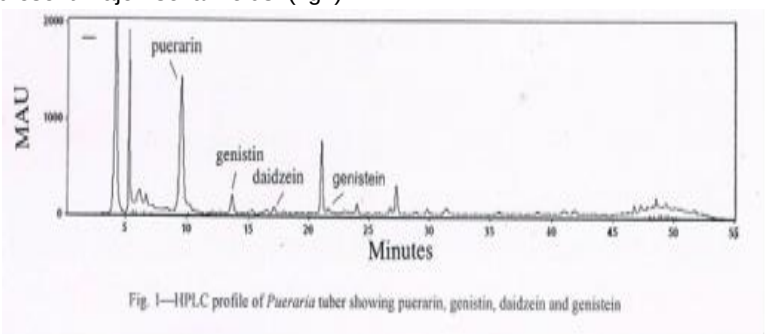


Fig. 1—HPLC profile of *Pueraria* tuber showing puerarin, genistin, daidzein and genistein

Cell Lines

HeLa[3,4] (cervical cancer) and MCF-7[5] (Breast cancer) Cell lines was obtained from CDRI Lucknow. The HeLa and MCF cells were cultured in DMEM 10% FBS complete medium. The medium wassupplemented with 10% heat inactivated fetal bovine serum, antibiotics. The celllines were maintained at 37° C in a 5% CO2 incubator and the media were changed frequently.

Sterility Test

Sterility test was done initially to check the extracts for the contamination. 35 mm culture dish was plated with the HeLa and MCF cell suspension in 2ml of DMEM media and allow the cells to adhere. Thecrude plant extracts were added into culture dishes and incubated at CO_2 incubator (5%) for 24 hours.

Cytotoxicity Test

Cytotoxicity test was done to check the drug activity of killing the cancerous cells. From the Helaand MCF cell cultured plate 1 ml of media was removed and then 1 ml of extract was added. After 24 hours incubation, the culture was observed under microscope and the morphological changes were identified.

Trypan Blue Test/ Dye Exclusion Test[6]

Cell suspension at a high concentration (approx10^6cells/ml) was prepared. Clean hemocytometer slide was taken and cover slip was fixed in place. 100 µl /well of cell suspensions (0.5-2.0×10^5cells/ml) were seeded in 96 well micro titer plates and incubated at 37°C to allow for cell attachment. The spent media was removed from each well. Drug mixed with media was added to each well and allowed to incubate for 24 and 48 hours. After 24 and 48 hrs, the contents of the well were emptied and 50 µl of trypan blue was added. After 30 sec, trypan blue was completely removed from the well and observed under microscope. Cells were counted. The number of stained cells and the total number of cells were counted. Viable cells exclude trypan blue, while dead cells stain blue due to trypan blue uptake. The IC50 (concentration of drug resulting in a 50% reduction in the counted cell number at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from:

[(Ti-Tz)/Tz] x 100 = -50,

Where Ti is the initial cell count and Tz is the final cell count. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested. IC 50 value was found with the obtained readings.

Test For Apoptosis Detection

Hoechst 33342 Dna Staining

It is possible to perform apoptosis detection assay with Hoechst 33342 (Sigma B-2262), but the increase in fluorescence seen in the apoptotic cells may be less dramatic. Hoechst dyes can also be obtained from Molecular Probes. H342 is a "vital" DNA stain that binds preferentially to A-T base-pairs. The cells require no permeabilization for labeling, but do require physiologic conditions since the dye internalization is an active transport process. This condition typically varies among cell types.

The procedure for staining and analysis of cells using Hoechst 33342 (H342)

Cell suspension: This procedure is very sensitive to cell concentration and pH of the media. Cells should be approximately 1- 2x10^6/ml, in buffered media, pH 7.2. It is also helpful to include 2% fetal calf serum to maintain the cells. Drug was added and incubated for 48 hours. Homogenously aspirated and spent media was removed and 1 ml of saline was added and centrifuged at 1500 rpm for 10 mins. Pellet was collected and 100 µl of Hoechststain was added to pellet. Cells were then incubated at 37^{o}C for 1 hour. Time is a critical factor due to the transport of the dye. Typically, 30 minutes is a minimum, but it is important to remember that the signal may begin to degrade after ~120 minutes. It is recommended that the staining kinetics be empirically defined. Analyze apoptosis unmicroscope after incubation. Washing is not recommended.

Test For Cellular Proliferation

Mtt Assay Protocol

Seeded the HeLa and MCF cells at 3 x 10^5cells/well in a 96 well plate. Cells may be seeded at different densities. At least three wells were left without cells. These wells serve as a control for the minimum absorbance. The plate was incubated overnight at 37°C in a humidified incubator, 5% CO_2 for the cancer cells to grow and adhere to the surface. Test compounds were added to the plate.

Include replicates for a range of concentrations. Include negative controls (including vehicle control) and a positive control. The final volume will be 100µl per well. The plate was incubated for overnight (or for some other appropriate time) at 37°C in a humidified incubator, 5% CO_2. MTT reagent (20µl/100µl per well of the 96 well plate) was added. Incubated at 37°C for 3 hours. 1 volume (100µl) of the stop mix solution was added and the plate was rocked at room temperature for a minimum of 1 hour. (Allows time for the formazan precipitate to dissolve).The stop mix solution must be added in a fumehood. A purple colour should be visible at this stage and should deepen over the 1 hour incubation period.

After the 1 hour incubation, ensure the formazan precipitate is dissolved by pipetting each well up and down until no precipitate is visible. Read the plate on a plate reader using wavelength at 572 nm. Tabulate results and calculate the % viability Mean Absorbance of Sample % Viability = x 100 Mean Absorbance of Control.

Notes: The area of a well is approximately 0.27cm^2. Use this factor to calculate numbers of cells for 48, 24 or 12 well plates if they are required. A vehicle control is required for each different solvent used to dissolve test compounds.

Results and Discussion

Trypan Blue Test/ Dye Exclusion Test der fluorescent. The trypan blue test results for 24 hrs, and 48hrs hrs incubation of crude drug for HeLa and MCF cells at the concentrations of 0.5 %, 0.45 %, 0.4 %, 0.35 %, 0.3%, 0.25%, 0.2%, and 0.15%. showed cytotoxic activity with the IC 50 concentration of drug is between 0.45% to 0.4% i.e 1.35µg/µl to 1.2µg/µl.

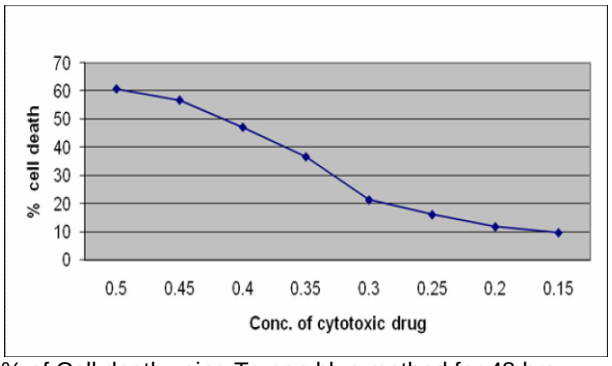
Hoechst 33342 Dna Staining

The leaf extract was found to be killing the cells by Apoptosis for both HeLa and MCF cells. Only few number of apoptotic cells were seen. Apoptotic cells are characterized by DNA fragmentation and cytoplasmic shrinkage.

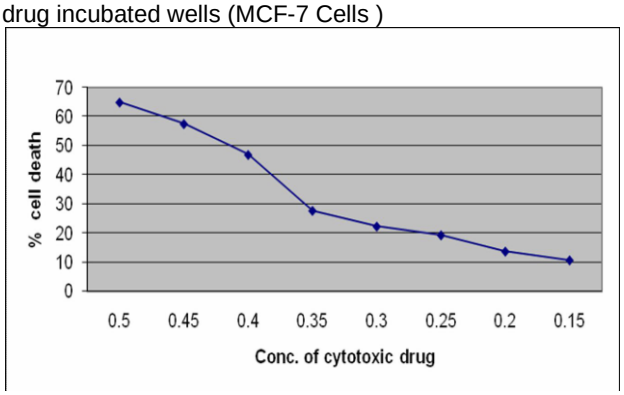
MTT Assay:

The MTT assay results for 24 hrs and 48hrs incubation of crude drug at the concentrations of 0.5%, 0.45%, 0.4%, 0.35, 0.3%, 0.25%, 0.2% and 0.15%. showed increased HeLa and Mcf cell viability as the concentration got diluted. % of Cell death using Trypan blue method for 24 hrs drug incubated wells (HeLaCells).

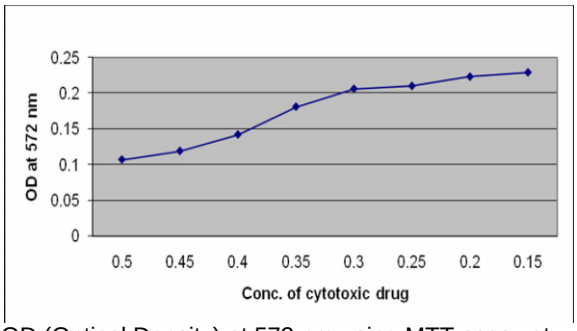
%of Cell death using Trypan blue method for 24 hrs drug incubated wells (HeLa Cells)



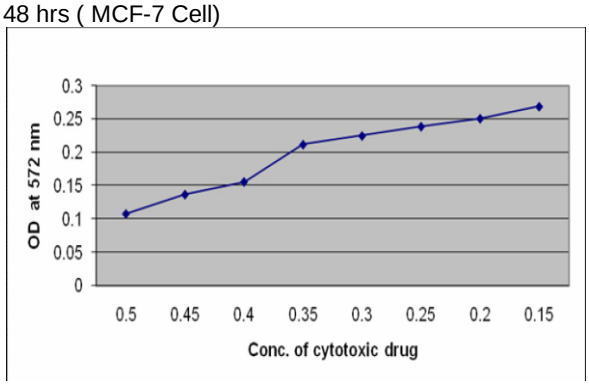
% of Cell death using Trypan blue method for 48 hrs drug incubated wells (MCF-7 Cells)



OD (Optical Density) at 572 nm using MTT assay at 48 hrs (HeLa Cell)



OD (Optical Density) at 572 nm using MTT assay at 48 hrs (MCF-7 Cell)



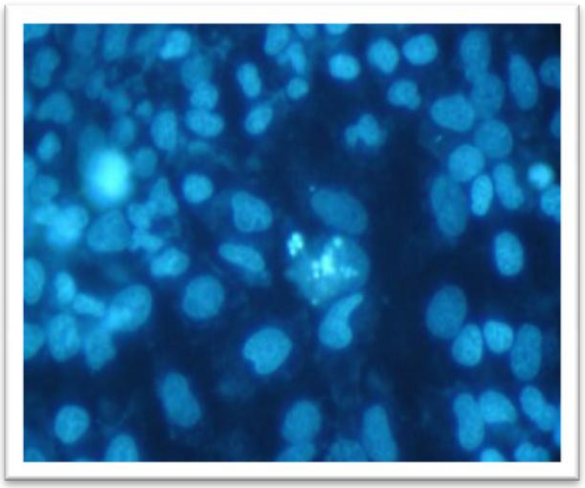
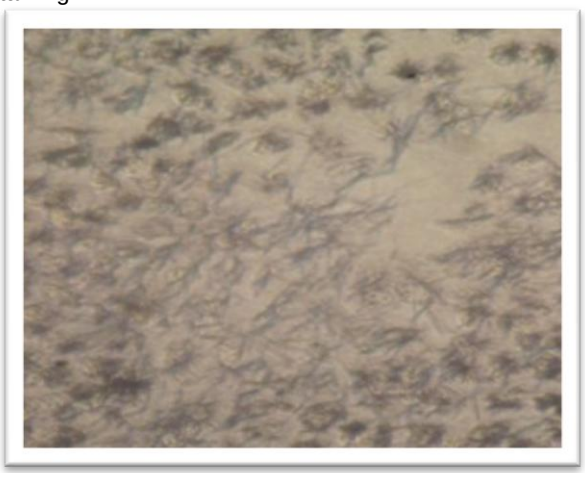


Fig.2 Necrotic and Apoptotic cells after Hoechst staining



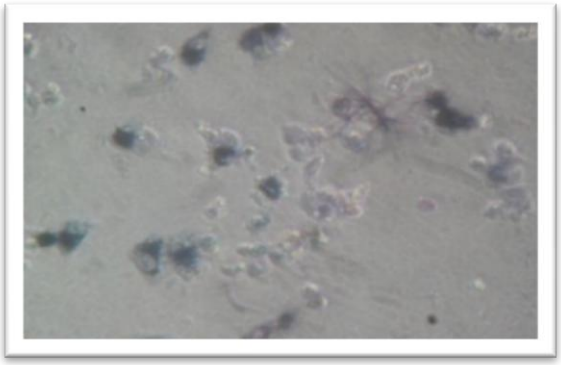


Fig.3 Crystal formation in vehicle controlFig.4Crystal formation at Methanolic extract

Acknowledgements

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