Research Paper

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Study of Anticancer Activity of Chlorophyllin from Phyllanthus Emblica L. Against Mcf-7 Cell Line

Science

KEYWORDS : Chlorophyllin, MCF-7 cell line, MTT, Phyllanthus emblica L., Anti-cancer

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ABSTRACT

The result of this study shows the anticancer activity of the medicinal plant of phyllanthus emblica L from the chlorophyllin content which is taken from leaf extract and purified. The purified chlorophyllin content has been taken to the anticancer activity of MCF-7 cell lines and compared with vero cell lines by MTT assay and result interpreted that the presence of anticancer activity in the phyllanthus emblica L. plant from the chlorophyllin content of leaf extract.

Introduction

Phyllanthus emblica is also known as emblic[1] and Indian gooseberry[2] is a deciduous tree of the family *Phyllanthaceae*. It is known for its edible fruit of the same name. Many preliminary research work and in vitro antiviral and antimicrobial properties work has been undergone in Indian gooseberry.[3] *Phyllanthus Emblica* is a natural plant used in folk medicine to treat a variety of ailments and for a number of applications. All parts of the plant are used in the herbal preparations, including the fruit, seed, leaves, root, bark and flowers, according to Wikipedia. It is used to stimulate hair growth by nourishing the hair and scalp and even preventing prematurely graying hair. The high tannin content of *Phyllanthus Emblica* was also used as a dye in inks, shampoos and hair oils. *Phyllanthus Emblica* may have the ability to regulate melanin production in the skin, reducing age spots and preventing photoaging and sun damage. Due to its high content of vitamin C and polyphenols, amla extract is a potent antioxidant. Antioxidative activity, polyphenolic content and anti-glycation effect of some thai medicinal plants traditionally used in diabetic patients. Med Chem. 2009.

MATERIALS AND METHODS

COLLECTION OF PLANTS

Phyllanthus emblica L., was collected in Unani hospital, Royapettah, Chennai.

EXTRACTION OF CHLOROPHYLLIN FROM *PHYLLANTHUS EMBLICA* (Schertz, 1928)

Ten grams of fresh leaves were taken and 1gm of sodium carbonate was added to neutralize the acidity. The plant material was ground with 50 – 100ml acetone and filtered using filter paper. This procedure is repeated until the residue becomes colorless. It was then washed with 50 – 150ml of diethyl ether to wash off acetone. The mixture was poured into a separating funnel and acetone was washed off using distilled water. This was repeated until a yellow color separates off which consists of flavones. The solution was poured into a bottle and 10 – 25ml of methanol saturated with potassium hydroxide pellets was added. The solution was shaken thoroughly and kept in icebox for overnight. The alkaline solution of chlorophyllin was poured into a separating funnel and 100ml diethyl ether was added and left for 30mins. Chlorophyllin separates off greenish layer which was removed. The ether layer was washed off with dilute potassium hydroxide and distilled water, to remove traces of chlorophyllin salts. The filtrate was evaporated to dryness in rotary evaporator and the extract was stored in ice box.

***In Vitro* STUDIES FOR CHLOROPHYLL AND CHLOROPHYLLIN**

COLLECTION OF CELL LINE

Breast Cancer Cell Line (MCF-7) and African Green Monkey Kidney (VERO) cell lines were obtained from National centre for cell sciences, Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

SUBCULTURING AND MAINTENANCE OF CELL LINE

The medium was brought to room temperature for thawing. The tissue culture bottles were observed for the growth, cell degeneration, pH and turbidity using an inverted microscope. If the cells (MCF-7 & VERO) attained 80% confluent it was taken for sub culturing process. The mouth of the bottle was wiped with cotton soaked with spirit to remove the adhering particles. The growth medium was discarded in a discarding jar. Then 4-5 ml of Minimum essential medium (MEM) without Foetal Calf Serum (FCS) was added and gently rinsed with titling. The dead cells and excess FCS was washed out and medium was discarded. TPVG was added over the cells and incubated a 37ºC for 5 min. for disaggregation. The cells disaggregate and becomes individual cells and is present as suspension. 5 ml of 10% MEM with FCS was added using a serological pipette. The process was repeated if any clumps were present. Then the cells were split into 1:2, 1:3 ratio for cytotoxicity studies by plating method.

SEEDING OF CELLS

The cell suspension was taken and poured into 24 well plate. In each well 1ml of the suspension was added and kept in a desiccators in 5% CO_2 atmosphere.

CELL OBSERVATION

After 2 days of incubation the cells were observed in an inverted microscope and photographs were taken (Olympus, Japan).

CYTOTOXICITY ASSAY

In order to study the anti tumor activity of a new drug, it is important to determine the cytotoxicity concentration of the drug. Cytotoxicity test defines the upper limit of extract concentration, which is non-toxic to the cell line. The concentration at which the drug is non toxic to the cells is chosen for anti cancer assay. After the addition of drugs, cell death and cell viability was estimated. The result was confirmed by additional metabolic intervention experiment such as MTT assay.

STOCK DRUG CONCENTRATION-100mg/ml

10µl of drug from stock was dissolved in 990µl of DMSO giving a working concentration of 1 mg/ml. The working concentration was prepared fresh and filtered through 0.5µl filter before each assay. 500µl of serum free MEM was taken in 9 eppendroff tubes. Then 500µl of this volume was transferred from first to last tube by serial dilution to obtain the desired concentration of drug. As a result the volume remains constant but there is a change in concentration. Each dilution of the drug ranges from 1:1 to 1:64.

SAMPLING

Forty eight hour monolayer culture of MCF-7 and VERO cells at a concentration of 1lakh cells/well were seeded in 24 well titer plate. The plates were microscopically examined for confluent monolayer, turbidity and toxicity if the cells become confluent.

The growth medium (MEM) was removed using a micropipette. Care was taken so that the tip of the pipette did not touch the cell sheet. The monolayer of cells was washed twice with serum free MEM to remove the dead cells and excess FCS. To the washed cell sheet , 1 ml of serum free medium containing defined concentration of the drug was added in the perspective wells. Each dilution of the drug ranges from 1:1 to 1:64 and they were added to the respective wells of the 24 well titer plates. To the cell control wells 1ml of serum free MEM was added. Every experiment included a set of negative control and positive control. The plates were incubated at 37˚C in 5% CO_2 environment and observed for cytotoxicity using inverted microscope.

MTT ASSAY

The tetrazolium salt 3, (4,5-dimethyl tiazol-2yl)-2, 5-diphenyl tetrazolium bromide is commonly known as MTT. It is a dye and is widely used in cytotoxicity assays. MTT assay was first proposed by Mossman, 1983.

PRINCIPLE

MTT is cleaved by mitochondrial dehydrogenase in viable cells, yielding a measurable purple colour product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity (Mosmann,1983).

PROCEDURE

After incubation the medium was removed from the wells carefully for MTT assay. Each well was washed with serum free MEM for 2-3 times. And 200µl of MTT was added and incubated for 6-7 hours in 5% CO_2 incubator for cytotoxicity. After incubation 1ml of DMSO was added to each well and mixed using a pipette and left for 45 seconds. If any viable cells were present the formazan crystals after adding solibilizing reagent (DMSO) showed purple colour formation. The suspension was then transferred to the cuvette of spectrophotometer and the OD values were red at 595 nm by taking DMSO as a blank. A graph was plotted by taking concentration of a drug on X-axis and relative cell viability on Y-axis. The percentage of cell viability was calculated using the formula

Cell viability (%) = Mean OD×100/control OD

RESULTS

This study results that phyllanthus emblica L. has anticancer activity from chlorophyllin against MCF-7 Cell line which has been compared with different concentrations and with the authentic chlorophyllin in Table 1 and Fig. 1. When compared with authentic chlorophyllin with cholorophylin extracted from phyllanthus emblica shows best result in the concentration of 150µg. Hence it showed the presence of anticancer activity against breast cancer cell line.

Table 1 : Table shows the concentration of viability and toxicity of chlorophyllin from *phyllanthus emblica* against MCF-7 Cell line

Concentration	Control	PC Cyclo-90 (90µg)	Chlorophyllin STD	Sample 50µg	Sample 100µg	Sample 150µg
% of Viability	100	27.3455	54.3478	60.6979	49.857	39.4451
% of Toxicity	0	72.6545	45.6522	39.3021	50.143	60.5549

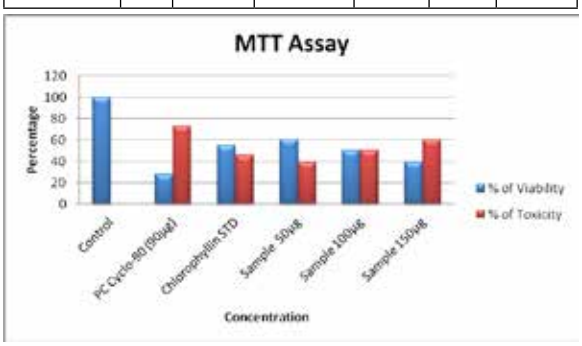

Fig. 1. Figure shows the anticancer activity of chlorophyllin from *Phyllanthus emblica L.* against MCF-7 Cell line

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