

Anticancer Activity of Phyllanthus Emblica Fruit Extract in Cancer Cell Lines

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ABSTRACT:

The present study was performed to investigate the anticancer activity of aqueous fruit extract of Phyllanthus emblica in human cancer cell lines. The cytotoxic effect was determined against HepG₂ (Liver cancer cell line) and HT-29 (Colon cancer cell line) using MTT assay. The aqueous fruit extract of Phyllanthus emblica exhibited higher cytotoxic activity in both HepG₂ and HT-29 cancer cell lines in a dose dependent manner when compared to the control. The findings of the present study thus indicate that Phyllanthus emblica possess potential anticancer activity.

Key Words: Phyllanthus emblica, anticancer activity, HepG₂ (Liver cancer cell line), HT-29 (Colon cancer cell line).

INTRODUCTION:

Cancer is a multifactorial as well as multimechanistic disease that requires a multidimensional approach for its treatment, control and prevention. It is a major health problem globally [1]. Over the last few decades, the incidence of cancer has increased with disproportionate rise in the cancer cases and deaths from the developing countries. The cancer when localized may be treated either with surgery or with ionizing radiation or by combining both the modalities. However in the advanced stage, when metastasis is observed, then the use of cytotoxic chemotherapeutic agents will become obligatory [2]. The use of chemotherapy and ionizing radiation produces toxic and deleterious side effects affecting the normal tissues [2,3]. Further, the treatment of cancer is very expensive and beyond the reach of most people in the developing countries. Thus, large number of people prefer alternative medicines for treating cancer [1].

Hepatocellular carcinoma (HCC) is considered to be the most common cancer worldwide occupying fifth position and also existing as a leading cause of death [4]. The occurrence of HCC is mostly associated with chronic infection due to Hepatitis B (HBV) and Hepatitis C (HCV) virus and also aflatoxins exposure [5,6]. Several effects have been made in search of natural materials and foods as a means of chemical prevention of liver cancer [7].

The colorectal cancer or the colon cancer occupies fourth position thus making upto 10% of all cases [8] and is more common in developed countries than in developing countries. About more than 1 million

people are reported with colorectal cancer every year[9]. The colorectal cancers are mostly caused due to life style factors and increase in age with only a small number of cases due to the underlying genetic disorders. Since diet plays an important role in the etiology of colon cancer, dietary chemo-prevention received attention for colon cancer prevention[10]. Ayurveda considered to be one of the oldest traditional Indian system of medicine is practised in the Indian subcontinent with an emphasis on disease prevention and promotion of good health by adopting a proper lifestyle and also following the therapeutic measures that will rejuvenate the body [11].

The Ayurvedic medicines which are preventive and therapeutic are mostly made of plants. The advantage behind the therapeutic use of the medicinal plants in various diseases is due to their safety besides being economical, effective and easy availability [12,13]. Over the past, the medicinal plant derived drug research has made tremendous progress in anticancer therapies. Many components of these medicinal plants have been identified to possess potential chemopreventive properties which are capable of either inhibiting, retarding or reversing the multistage process[14,15]. India is bestowed with a number of medicinal plants. One such plant is *Phyllanthus emblica* or *Embolica officinalis*. The plant is highly regarded as significant due to rich vitamin C content [16]. Almost all parts of *Phyllanthus emblica* tree are used in treating several ailments but the fruit is of immense use in various folk and traditional system of medicine[17-19]. Since generations, the extract from the fruit has been used to treat the symptoms ranging from constipation to the treatment of tumors[20].

The Amla fruit is reported to possess broad range of therapeutic effects that includes anticarcinogenesis and antimutagenic[21], antitumor [22] and induction of apoptosis[23]. Keeping in view the above facts, the potential anticancer activity of aqueous fruit extract of *Phyllanthus emblica* was tested in human cancer cell lines. i.e. HepG₂ (Liver cancer cell line) and HT-29 (Colon Cancer cell line).

HepG₂ is an immortal cancer cell line derived from the liver tissue[24]. These cell lines are a suitable in-vitro model system for the study of human hepatocytes. The HepG₂ cells serve as a suitable model to study the intracellular trafficking and dynamics of membrane proteins and lipids in vitro [25] due to their high degree of morphological and functional differentiation. Further, the HepG₂ cells are also used to study human liver diseases and also as a model system to study the metabolism of liver and toxicity of xenobiotics[26]. These cells are also used in trials with bioartificial liver devices.

HT-29 is a human colon cancer cell line which is used widely in biological and cancer research [27]. HT-29 cells have been studied for their ability to differentiate and simulate the real colon tissue in vitro. This characteristic feature of HT-29 cell lines made it useful for epithelial cell research[28]. Further, the HT-29 cells can also be tested in vivo via the Xenografts using rodents.

The present study demonstrates the anticancer activity of aqueous fruit extract of *Phyllanthus emblica* in human cancer cell lines (HepG₂ & HT-29).

MATERIALS AND METHODS:

Dulbecco's modified Eagler medium (DMEM), MTT (3-(4,5-dimethylthiazol-2-yl) -2,5- diphenyl tetrazolium bromide) and trypsin, EDTA and phosphate buffered saline (PBS) were purchased from Sigma Aldrich (St. Louis, MO, USA) and fetal calf serum (FBS) was purchased from Gibco. Whatman filter paper no- 42, a 75 cm² flask and 96 well tissue culture plates were purchased from Eppendorf, India.

Preparation of Aqueous Fruit Extract of Phyllanthus Emblica:

Fresh fruits of Phyllanthus emblica were purchased from the local market. The fruits were then washed, deseeded, air dried and powdered with a mechanical grinder passing through the sieve and stored in a tight container. About 25 gms of the air dried fruit powder is refluxed with ethanol at 45⁰C for 3 hours using soxhlet apparatus. The mixture was filtered and the filtrate was evaporated using vacuum rotary evaporator and air dried at 40⁰C. Then stock solution of the crude ethanolic extract (aqueous extract) was prepared by diluting the dried extract with 0.25% dimethyl sulphoxide solution (DMSO) to obtain a final concentration of 100mg/ml.

Cell Culture: HepG₂ and HT-29 cell lines were procured from the National centre for cell science (NCCS), Pune, India and were thawed by gentle agitation of their containers i.e vials for 2 minutes in a water bath at 37⁰C. After thawing, the content of each vial was then transferred to a 75cm² tissue culture flask diluted with DMEM supplemented with 10% FBS and 1% penicillin and streptomycin and was later incubated for 2 to 3 days at 37⁰C in a 5% CO₂ incubator. The growth medium was changed twice a week. The cells grown to 75-85% confluence were washed with phosphate buffer saline (PBS), then trypsinized with 3ml of 0.25% (V) trypsin, -0.3% (V) EDTA, diluted with fresh medium and counted using haemocytometer.

MTT Assay: The anticancer activity of aqueous fruit extract of Phyllanthus emblica was assessed using MTT assay[29] in human HepG₂ and HT-29 human cancer cell lines. The assay is based on the reduction of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl -2H tetrazolium bromide) by mitochondrial dehydrogenase to purple formazon. To conduct this assay, the cells at a density of 1 × 10⁴ cells/ml were plated in 96 well tissue culture plates and incubated for 24hrs to allow for cell attachment separately for both HepG₂ and HT-29 cancer cell lines. Then the cells were treated with fresh medium containing different concentrations (10 to 100µg/ml) of aqueous fruit extract of Phyllanthus emblica dissolved in DMSO and then incubated for 48 hours at 37⁰C. The control group (Cisplatin) received same amount of DMSO. At the end of 48 hours incubation, about 200µl of MTT solution was added and incubated for another 4 hours at 37⁰C. Then the supernatant was removed and replaced with 500µl of DMSO to dissolve the resulting MTT formazon crystals followed by incubation for 10min to dissolve any air bubbles. The culture plate was then placed on a Biotek microplate reader and the absorbance was measured at 590nm. The percentage (%) cell viability and percentage (%) cytotoxicity were calculated.

Cell Viability = O.D of Sample – O.D of Control/O.D of Control × 100.

Where O.D = Optical density.

STATISTICAL ANALYSIS: Multiple linear regression analysis was used for the comparison of data through Statistica version (5.0) (Statsoft, Hyderabad, India) and the results are expressed as Mean ± S.D. The value of P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION: The anticancerous activity of aqueous fruit extract in HepG₂ and HT-29 cancer cell lines using MTT assay are shown in Table-I and Figure-I (A & B) respectively. The aqueous fruit extract of *Phyllanthus emblica* exhibited higher cytotoxic activity in both HepG₂ and HT-29 cancer cell lines in a dose dependent manner when compared to the control (Cisplatin).

TABLE- I

Anticancer activity of Aqueous fruit extract of *Phyllanthus emblica* and Cisplatin in HepG₂ and HT-29 Cancer cell lines.

Sample	Concentration in µg/ml	(%)Cell Viability (HepG ₂ cell line)	(%) Cell Viability (HT-29 cell line)
Aqueous fruit extract of <i>Phyllanthus emblica</i>	20	43.20±1.76*	45.92±3.22*
	40	55.87±2.25*	58.27±2.01*
	60	67.19±0.40*	68.78±0.74*
	80	77.53±0.95*	78.50±1.99*
	100	79.20±1.99*	82.68±1.81*
Cisplatin(Standard)	20	45.92±3.21	47.78±2.66
	40	61.13±1.66	62.89±0.70
	60	70.50±1.99	71.53±0.95
	80	80.54±2.01	81.13±1.66
	100	82.89±2.26	84.20±1.20

Values are expressed as Mean± S.D. * P<0.05 compared to standard.

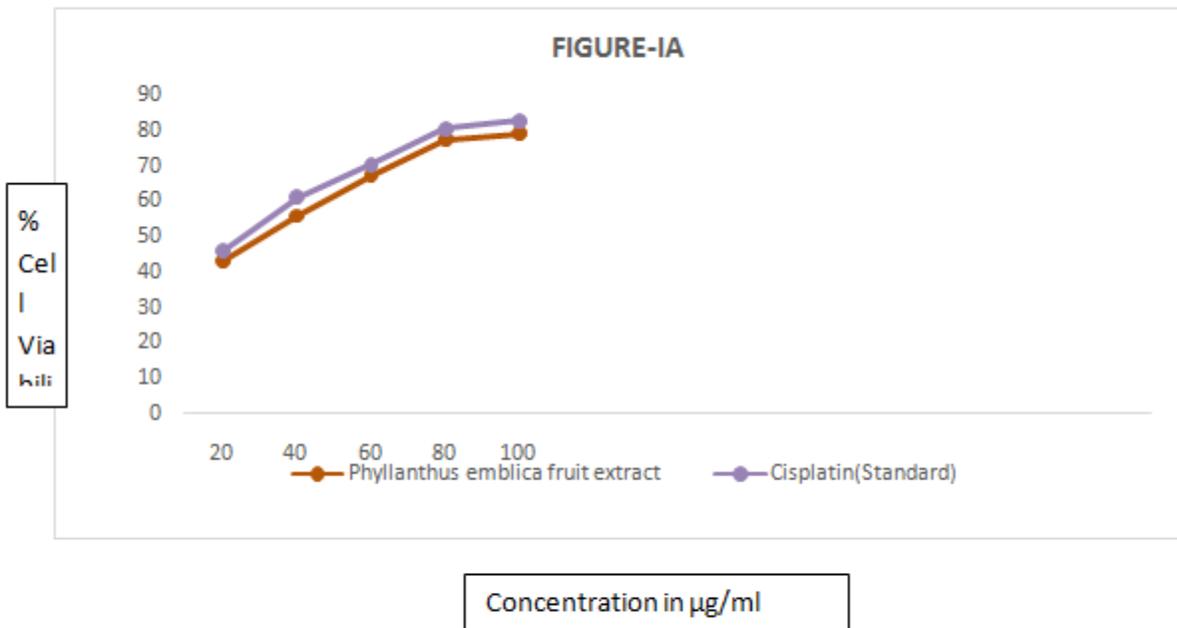


Figure-1A Anticancer activity of Aqueous fruit extract of Phyllanthus emblica in HepG₂ Cancer cell lines

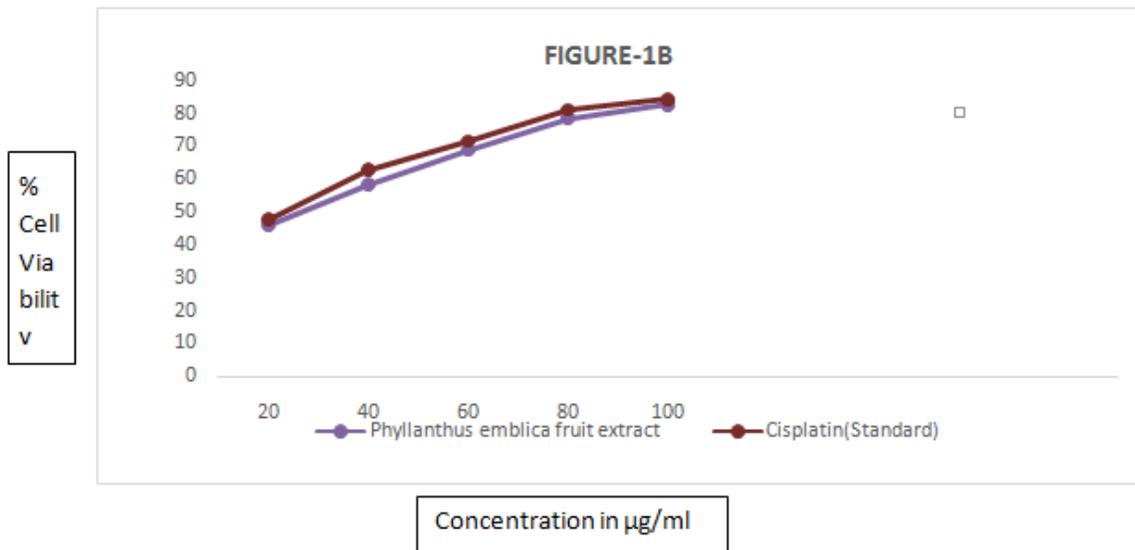


Figure -1B Anticancer activity of Aqueous fruit extract of Phyllanthus emblica in HT-29 Cancer cell lines

It is found that the incubation of cancer cell lines (HepG₂ and HT-29) with aqueous fruit extract of *Phyllanthus emblica* reduced the viability of these cells and significantly increased the dead cells with high concentration. The aqueous fruit extract of *Phyllanthus emblica* exhibited high cytotoxicity in both the cell lines in concomitant with the earlier results as observed by [30,31]. Even at a very low concentration, aqueous fruit extract of *Phyllanthus emblica* showed about 15 to 20% of dead cells. *Phyllanthus emblica* fruit extract significantly inhibited the growth of several human cancer cell lines as reported by Ngamkitidechakul, et al [32]. The fruits of *Phyllanthus emblica* were proved to be beneficial to treat different types of cancers as revealed by several research reports. The findings of the present study thus indicate that *Phyllanthus emblica* fruit possess potential anticancer activity.

CONCLUSION: The results of the present study highlight the effectiveness and potential application of aqueous fruit extract of *Phyllanthus emblica* in the treatment of cancer. The study supports the anticancer property of medicinal plants that are used in the traditional Indian system of medicine with further evaluation of the medicinal plants for an effective anticancer drug with minimal side effects. Hence, supplementation of balanced diet with the fruit of *Phyllanthus emblica* may have more beneficial effect.

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