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Research Article

IN VITRO CYTOTOXICITY OF EMBLICA OFFICINALIS AGAINST DIFFERENT HUMAN CANCER CELL LINES

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ABSTRACT

Cancer is a public health problem all over the world. Large number of plants and their isolated constituents have been shown to potential anticancer activity. Ethanolic whole plant extract of *Emblica officinalis* (syn. *Phyllanthus emblica* L.) showed *in vitro* cytotoxicity against different human cancer cell lines such as lung, neuroblastima, and colon. There was no growth of inhibition recorded against liver cancer cell line. Sulforhodamine B dye (SRB) assay was done for *in vitro* cytotoxicity test assay. The *in vitro* cytotoxicity was performed against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastima (IMR-32). The activity was done using 100µg/ml of the extract. Against lung (A-549) cell line plant extract showed 82% growth of inhibition. In case of liver (Hep-2) showed no activity reported, where as in case of colon 502713 cell line plant extract showed maximum activity. In case of HT-29 liver human cancer line and IMR-32 neuroblastima cell line plant extract showed 98% and 97% activity respectively.

Keywords: Human cancer cell lines, *in vitro,* cytotoxicity test, SRB, *Emblica officinalis*

INTRODUCTION

Emblica officinalis (syn. *Phyllanthus emblica* L.) is used therapeutically in Indian system of medicine. Fruits of E.O are used for the treatment of a number of diseases (Nadkarni, 1952; Chopra *et al.,* 1958) and is a constituent of many hepatoprotective formulations available (Antarkar *et al.,* 1980; De *et al.,*1993). It is an antibacterial and anti inflammatory agent (Asmawi *et al.,* 1993) and modifies metal induced clastogenic effects (Dhir *et al.,*1990). In the present communication, we report its *in vitro* cytotoxicity activity against different human cancer cell lines.

EXPERIMENTAL

Plant material

Emblica officinalis was collected in February to March 2009 from Uttarakhand. The whole plant ethanolic extract was used for *in vitro* cytotoxicity properties.

Preparation of plant extracts

Plant material was dried at 37^{0}C, powdered and extracted in ethanol. Extract was fine-filtered and freeze dried. For the preparation of the extracts, dried ground plant material was percolated with 95% ethanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing on cell lines.

Human cell lines

Human cancer cell lines namely of lung (A-549), liver (hep-2) colon (502713 HT-29) and neuroblastima (IMR-32) were grown in RPMI-1640 with 2 mM L-glutamine medium pH 7.2. Penicillin was dissolved in PBS and sterilized by filtering through 0.2µ filter in laminar air flow hood. The media was stored in refrigerator (2-8^{o}C). Complete growth medium contains 10 % FCS. The medium for cryopreservation contains 20 % FCS and 10 % DMSO in growth medium. The cell lines were maintained at 37^{0}C in a 5% CO_2 atmosphere with 95% humidity.

In vitro assay for cytotoxic activity

The anticancer activity was determined by the cytotoxic potential of the test material using human cancer cell lines which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of Cell suspension for assay

Human cancer cell lines were grown in multiple tri conical flasks (TCFs) at 37^{0}C in an atmosphere of 5% CO_2 and 90% relative humidity in complete growth medium to obtain enough number of cells. The flasks with cells at subconfluent stage were selected. Cells were harvested by treatment with Trypsin-EDTA. Cells were separated to single cell suspension by gentle pipetting action and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000 - 40,000 cells/100µl depending upon the cell line Monks (1991). 100µl of cell suspension together with 100µl of complete growth medium was added into each well. The plates were incubated at 37^{0}C for 24 hours in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. After 24 hours, the test material, DMSO (vehicle control) and positive control were added.

Sulforhodamine B (SRB) assay

The antiproliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye Skehan (1990). The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

RESULTS & DISCUSSION

Plants are storehouse of good variety of compounds. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of Anticancer activity agents (Satish et al.,2010).The test sample showing growth inhibition more than 70% at 100µg/ml is considered to be active. The *in vitro* cytotoxicity was performed against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713 HT-29) and neuroblastima (IMR-32). Against lung (A-549) cell line plant extract showed 82% growth of inhibition. In case of liver (Hep-2) showed no activity. Where as in

case of colon 502713 cell line plant extract showed maximum activity. In case of HT-29 liver human cancer line and IMR-32 neuroblastima cell line plant extract showed 98% and 97% activity respectively. Fruits of E.O are used for the treatment of a number of diseases (Nadkarni, 1952; Chopra *et al.,* 1958) and is a constituent of many hepatoprotective formulations available (Antarkar *et al.,* 1980; De *et al.,*1993). It is an antibacterial (Godbole and Pendse, 1960) and antiinflammatory agent (Asmawi *et al.,* 1993) and modifies metal induced clastogenic effects (Dhir *et al.,*1990). Chyavanprash, a drug preparation in which E.O is a major component is widely used as a health tonic. It is claimed to reduce aging and age-related ailments (Ojha,1988).Preparation of chyavanaprash involves making of decoction from 35 herbs, of which many are known immunomodulatory and antioxidants and made into a past with brown sugar and taste with species. E.O was shown to be potent free radical scavenging agents (Jeena and Kuttan, 1995), thereby preventing carcinogenesis and mutagenesis (Jeena *et al.,* 1997, 1999).

In the present study, we conclude that the plant extracts showed selective *in vitro* cytotoxity, active against some human cancer cell lines and other not showed activity. Activity depends upon the morphology and mechanisim of action plant extract. Many plant extract kill the cancer cell lines through activating apoptosis and some through effecting growth regulators. Other than anticancer activity this plant extract also show the other activity.It is not possible at this juncture to single out the most effective *in vitro* cytotoxicity constituent of plant. However, based on the published studies alkaloids seem to be most likely candidates eliciting *in vitro* cytotoxicity effect. Its reported *in vitro* cytotoxicity effects warrant further investigation for its use in the cases of clinical anticancer activity.

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