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Original article

In vitro cytotoxic and apoptotic effect of *Mimusops elengi* Linn. methanolic bark extract

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ABSTRACT

The ayurvedic system of medicine recommends *Mimusops elengi* Linn. (Sapotaceae) for the treatment of tumors. The study is needed as cancer is the second most common cause for death in the world; most of the traditional drugs have shown resistance, low-cost approaches, and higher toxicity of the existing compound. The present study aims to determine the mode of cell death induced by the methanol extract of ME in human cancer cell lines. Among *M. elengi* (ME) crude extract and fractions screened for potential anticancer activity, MED and MEE fractions (dichloromethane and ethyl acetate) were found to have promising cytotoxic activities in cell lines studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and Sulforhodamine B (SRB). MEE found to have more potent activity in cervical cancer (HeLa), colon cancer (HCT15), lung (A549), breast cancer (BT549), glioblastoma (U343 cells). Interestingly ME was less cytotoxic in normal cell line (HBL-100) indicate the specific activity towards cancer cells. AO/EB staining and Hoechst-33342 staining indicate membrane blebbing, condensed and fragmented nuclei upon treatment with MEE and MED in HeLa, A549 and U343 cells. Fragmented DNA ladder and genomic DNA fragmentation were observed with DNA fragmentation assay based on gel electrophoresis and COMET assay by fluorescent microscopy. Similarly, cell cycle analysis by flow cytometer indicates distortion of normal cell cycle and increased subG$_0$ phase. ME was found genotoxicity micronuclei formation. These results indicate that both MED and MEE fractions induces apoptosis but not necrosis cells in the cancer cells.

1. Introduction

Apoptosis is cellular suicide or programmed cell death that is mediated by activation of an evolutionary conserved intracellular pathway. Recently the relation of apoptosis and cancer has been emphasized and increasing evidence suggests that the processes of neoplastic transformation, progression and metastasis involve alterations of normal apoptotic pathway [1]. Apoptotic also gives some clues about effective anticancer therapy and many chemotherapeutic agents were reported to event their anti-tumor effect including apoptosis of cancer cells.

Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value [2]. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action on the human body.

Mimusops elengi Linn. (Family – Sapotaceae) ("Syn.": Bakul, Maulsari) is commonly known as Spanish cherry or bullet wood is a small to large tree found in all parts of India. It is an evergreen tree whose distribution extends to India, Burma, Pakistan and Thailand. The plant has been used as febrifuges, astringents, headache, cardiotonic, alexipharmic and stomachic, anthelmintic, teeth cleaner and purgatives and stimulants [3]. The various extracts of the plant have been reported (bark, fruit, leaves, seed, flowers) to have antimicrobial, anti-ulcer, anti-hyperglycemic, diuretic, wound healing, anti-inflammatory, analgesic and antipyretic, antigastric ulcers, hypotensive, anti-HIV and spasmolytic activities.

Recent reports suggest that bark of the plant contains presence of steroids, alkaloids, taraxerol, taraxerone, urosolic acid, betulinic acid, α-spinosterol, β-sitosterol glycoside, quercitol, lupeol, β-amyrin, farnane type triterpenoid and mixture of

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triterpenoidsaponins in the bark of *M. elengi* Linn. have been isolated from the bark such as mimusopgenone and mimugenone in the seeds [4], triterpenoid saponins, such as mimusopsides A and B, mimusopin, mimusopsin, mimusin, Mi-saponin A and 16α-hydroxyMi-saponin A from the seeds [5,6]. Members of the lupane, ursane and oleanane triterpenoids have demonstrated antiproliferative activity on various cancer cell lines.

2. Materials and methods

2.1. Collection of plant material

The fresh plant bark was collected from parkala (Udupi), Karnataka in September month and authenticated by Dr GopalakrishnaBhat, Professor of Botany, PoornaPrajna College, Udupi, Karnataka. An authenticated specimen was submitted in herbarium of Manipal college of Pharmaceutical sciences, Manipal.

2.2. Preparation of extract

The fresh plant bark was harvested, rinsed under tap water and oven dried. The coarsely powder material was extracted with methanol by using soxhlet apparatus and solvent was removed by distillation and concentrated using a rotavapor.

2.3. Fractionation of crude extract

Crude methanolic extract of *M. elengi* Linn. was suspended in water and then fractionated with organic solvents in order of increasing polarity to get petroleum ether, dichloromethane, n-butanol, ethyl acetate and methanol-water (hydro-alcoholic mixture) [7].

2.4. Preparation of different concentrations

Different concentrations of crude extract and fractions were prepared by dissolving the extract in DMSO and then diluting with DMEM medium under sterile conditions.

2.5. Cell culture conditions

A cell line was procured from NCCS, Pune. Cancer cells were maintained in Dulbecco's modified eagle medium (DMEM) with 1000 mg/mL of glucose supplemented with 10% FBSFBS (fetal bovine serum) and penicillin/streptomycin-L-glutamine and cultured in a humified atmosphere of 5% CO_2 and 95% air at 37 °C in incubator [8].

2.5.1. Screening of cytotoxicity/anticancer potential

Cancer cell line was used for the determination of cytotoxic activity. Cells were seeded in 96-well plates at the density of 6000 cells/well (HeLa cells) in 100 μL medium. Then various concentrations of the crude extract were added to the cells in 100 μL medium. Cells were incubated for 24 h with test extract concentrations. Each concentration was tested in triplicate.

The MTT assay is a laboratory test that measures changes in color for measuring the activity of enzyme that reduces MTT to formazan, giving a purple color. Yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) reduce to purple formazan in living cells [9]. After 48 h incubation, 20 μL (5 mg/mL) MTT reagent was added to each well and incubated for additional 4 h. Then 200 μL of DMSO solution was added to each well to solubilize the formazan crystals. The plates were read for optical density at 540 nm with reference 630 nm, using a plate reader. By using optical density, percentage inhibition of was calculated.

The cytotoxicity assay was performed by using the Sulforhodamine B (SRB) colorimetric method to assess growth inhibition according to Vanicha and Kirtikara [10]. Briefly, till 48 h treatment same as that of MTT method. At the end of the exposed time, cells in each well were fixed by addition of 100 mL of cold [(4 °C) 10% (w/v)] trichloroacetic acid (TCA) into the growth medium. Each plate was incubated at4 °C for 1 h before gently washed five times with Mili-Q water to remove TCA, the growth medium and dead cells. Plates were allowed to dry in air and to each well were added 50 mL of 0.057% (v/v) SRB dye in 1% acetic acid in deionized water and allowed to stand for 30 min at room temperature. At the end of the staining period, unbound SRB was removed by washing four times with 1% of an acetic acid solution. The [illegible] was air-dried and

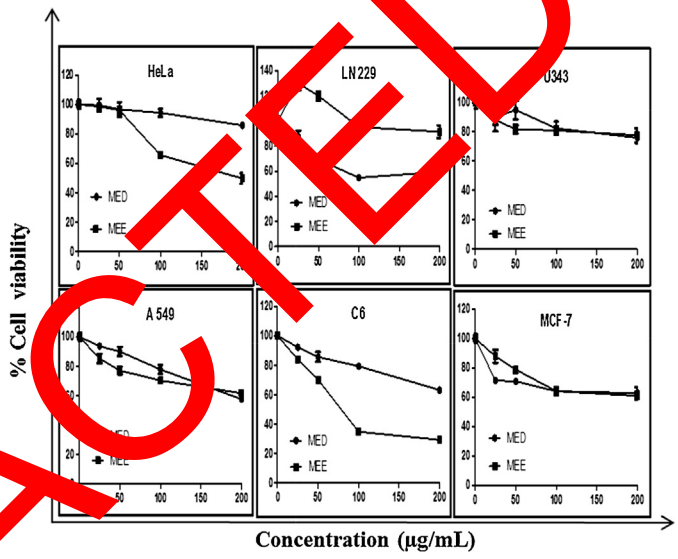

Fig. 1. Cytotoxicity of most active fractions in selected human cancer cells (MTT Method). 3A: human cervical carcinoma (HeLa), 3B: human glioblastoma cells (LN-229), 3C: human glioblastoma cells (U343), 3D: human adenocarcinoma alveolar basal epithelial cells (A549), 3E: rat glioma cells (C6), 3F: human breast adenocarcinoma epithelial cells (MCF7).

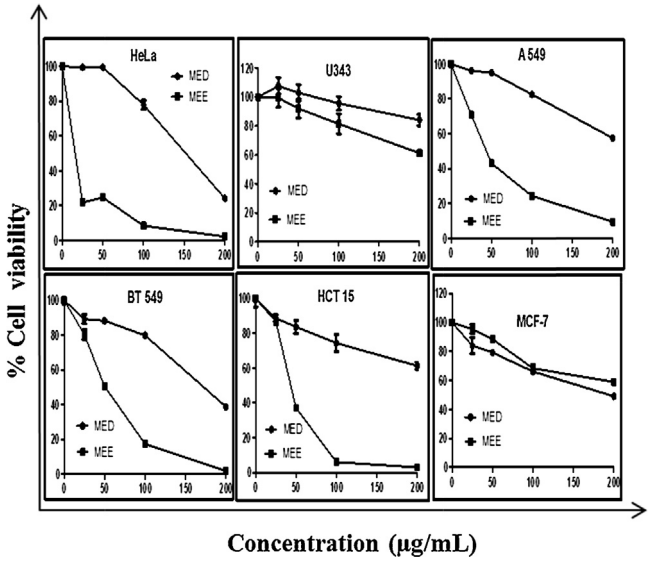

Fig. 2. Cytotoxicity of most active fractions in selected human cancer cells (SRB Method). A: human cervical carcinoma (HeLa), B: human glioblastoma cells (U343), C: human adenocarcinoma alveolar basal epithelial cells (A549), D: human breast ductal carcinoma cells (BT-549), E: human colorectal adenocarcinoma (HCT-15), F: human breast adenocarcinoma epithelial cells (MCF7).