COMPARATIVE ANTICANCER EVALUATION OF SELECTED MEDICINAL PLANTS

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Abstract

Cancer is an ailment which gradually influences and brings down the life expectancy of human begins. There is an increasing exigency towards the aversion and remedy for this life threatening disease. Fortunately, most of the researches accentuate on naturally derived product, as they considered having less toxicity and less side effects when compared to the current treatment viz., chemotherapy. Actually, the most prevalent drug sources are natural drugs and can provide more structural diversity and novelty. So in the present study, the anti-proliferative potential of extracts of three Indian medicinal plants namely *Ammania baccifera* ,*Azima tetracantha*., *Melothria maderaspatana* were evaluated against human cervical cancer cell line (Hela) by cytotoxic assay (MTT). The results of cellular viability in human cervical Cancer line (Hela) demonstrated a pronounced anti proliferative activity that may be attributed to the phyto constituent present in the extracts.

Introduction

Cancer, after coronary artery disease, is the second leading cause reduces the life expectancy of both men and women. All over the world, stupendous amount of resources are being put together in prevention, diagnosis and treatment of the life threatening cardiac vascular-disease.

Nowadays, there is a huge advancement in Chemotherapy as a lot of anticancer drugs have been recognized for the past few decades. Considering its unacceptable side effects, researches started to focus on natural drugs as it is a most prevalent drug sources and provide more structural diversity. In fact, the anti-tumour activity of natural products have been linked to their ability to trigger cell death Pathways including apoptosis in cancer cell. So the compounds that exerts a direct action on mitochondria of the cancer cell, present promising experimental cancer therapeutics, since they trigger cell death under certain circumstances in which standard chemotherapeutics fail, thus opening new perspective to overcome some forms of drug resistance

Methods

Evaluation of Anticancer Activity

The anticancer activity of the hexane and ethanol extracts of all the three plant drugs were evaluated using the cytotoxic assay, MTT assay, which is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5 dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) into an insoluble, coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells and the level of activity is a measure of the viability of the cells[1,2].

In Vitro Anticancer Screening Studies Using HeLa Cell Line[3,4]

The human cervical cancer cell line (HeLa) was obtained from National Center for Cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).

For Screening experiment the cells were seeded into 96 well plates in 100 µl of the medium containing 10% FBS, at planting density of 10,000 cells /well and incubated at 37°C ,5% CO_2,95% air and 100% relative humanity (RH) for 24h prior to addition of extracts. ALL the six extracts (ABE, ABH, MME, MMH, ATE and ATH) were solubilised in dimethyl sulfoxide (DMSO) and diluted in respective serum free medium. After 24h, 100µl of the medium containing the extracts at various concentrations (62.5µg/ml,125µg/ml,250µg/ml,500µg/ml and 1000µg/ml) was added and incubated at 37°C, 5% CO2, 95% air and 100% RH for 48h. Triplicate was maintained and the media without extracts were taken as control cells were also inoculated with the standard drug tamoxifen at a concentration of 10µg/ml. After 48h, 15µl of MTT (5µg/ml) in phosphate suffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was solubilised in 100µl of DMSO and then absorbance was measured at 570nm using micro plate reader, The percentage cell inhibition was determined using the following formula and IC50 values were calculated from the graph plotted between percentage cell inhibition and log concentration.

Percentage cell inhibition = [(100- Abs (drug)/Abs (control)] X 100

Results:

The percent cell growth inhibition by each extract was studied on HeLa cell line by MTT cell viability assay.

Treatment of HeLa cells with all the extracts resulted in growth inhibition and the percentage cell inhibition at various concentrations are tabulated in Table I.

The graphical representation of % growth inhibition are given in Fig1-6 and IC_{50} values are also given in Table I Positive control (Tamoxifen) showed the highest percent cell inhibition as it is a well known anti-estrogenic drug which attacks cervical cancers on several fronts when used at high dose for short term[5].

Among the six extracts tested, *Azima tetracantha* hexane extract was the most active against HeLa cell line as shown by the least IC_{50} value (130mg/ml). The pattern of cell growth inhibition was found to be minimum at lower concentrations tested (0.063 and 0.125mg/ml) followed by a sudden rapid increase and almost 100% inhibition was noticed at the maximum dose tested (100mg/ml) for all extracts except *Azima tetracantha* hexane extract for which 95% inhibition resulted with 250mg/ml itself and 100% inhibition attained at 500mg/ml.

The hexane extracts of *Melothria maderaspatna* and *Azima tetracantha* were better in inhibiting cancer cell growth compared to ethanolic extact, whereas ethanolic extract of Ammania baccifera showed greater anticancer activity than that of its hexane extract.

Discussion:

Phenolic compounds exhibit a prominent role in the prevention of cancer as well as anti proliferative and cytotoxic action in several tumour cells [6].

The reason for the absence of a linear correlation between the phenolic content and the invitro antiproliferative acitivity of the extracts may be the contributed by the other classes of phytoconstituents which possess anti cancer activity.

The other major classes of phytoconstituents, steroids and saponins, present in the extracts, have also been reported for anti proliferative activity through the induction of tumour cell apoptosis and changes in membrane permeability and pore formation [7,8,9].

Betulinic acid was isolated from the hexane extracts of all the three selected plants. Betutinic acid a widely distributed pentacyclic lupine – type triterpene thas been identified as a highly selective growth inhibitor of human melanoma, neuro ectodermal and malignant tumour cells and was reported to induce apoptosis in these cells[10]. It has proved active in vitro against a panel of neoplastic cell lines including cervical carcinomas[11]. Betulinic acid holds great promise as a novel therapeutic strategy in the treatment of human cancers as it induces apoptotic cell death in cancer cells by triggering the mitochondrial pathway of apoptosis, a mechanism useful in the failure of standard chemotherapeutics[12]. The effect of betulinic acid on mitochondrial functions results in antiangiogenic effects in endothelial cells and betulinic acid has also been reported to inhibit amino peptidase N, an enzyme that is involved in the regulation of angiogenesis and over expressed in several cancers [13]. Betulinic acid, a triterpenoid, present in *Ammania baccifera* root (1636.4ppm) may be responsible for the exhibited anti cancer activity [14].

Another phytoconstituent isolated and quantified from the hexane extracts of all the three plants is β sitosterol, a phytosterol the dietary inclusion of which has been proved to enhance tamoxifen efficacy in breast cancer patients[15]. β sitosterol, a constituent of vegetable oils has been claimed for reducing the breast cancer risk[16] and it has shown anticancer activity in cervix cancer cells[17].

Quercetin, a flavonoid isolated from *Azima tetracantha*, a potential anticancer agent the mechanism of action of which including cell cycle regulation, interaction with type II estrogens binding sites, and tyrosine kinase inhibition [18]. Quercetin when administrated by short i.v infusion in a phase I clinical trial has demonstrated tyrosine kinase inhibition in vivo also [19]. Earlier reports on anticancer activity of plant drugs reveal the cytotoxic potential of the chosen plants.

Ethanolic leaf extract of *Azima tetracantha* lam has been reported to possess significant anticancer activity against Ehrlich Ascites Carcinoma Tumour bearing mice[20]. The present study also demonstrates the maximum antiproliferative acitivity of *A.Tetracantha* hexane extract.

The methanolic extract of *Ammania baccifera* leaves displayed selective cancer cell line cytoloxicity with IC_{50} values of 0.55, 0.59 and 0.91 mg/ml-1 against gastric, colon and breast cancer cells respectively[21]. The present study confirms the cytotoxic activity of *Ammannia*

baccifera on HeLa cell line also. *Melothria madarapattana* has also been reported to possess anticancer activity [22].

Table:1

Effect of ethanolic and hexane extracts of *Ammania baccifera*, *Melothria maderaspatna* and *Azima tetracantha* on the percent cell inhibition of Hela cell line in all in intro anticancer assay.

S.No	Name of the extract	IC_{50}(µg/ml)	Dose mg/ml					
			0.063	0.125	0.25	0.5	1	Percent cell inhibition
1	*Ammania baccifera* hexane	270	3.91	14.45	37.97	95.91	99.88	
2	*Ammania baccifera* ethanolic	170	4.40	25.74	76.86	87.16	98.45	
3	*Melothria maderaspatna* hexane	250	7.94	22.02	39.02	95.41	99.75	
4	*Melothria maderaspatna* ethanolic	440	3.16	7.88	23.45	50.68	94.73	
5	*Azima tetracantha* hexane	130	17.10	37.63	95.08	100	100	
6	*Azima tetracantha* ethanolic	330	8.03	18.13	27.72	71.96	100	
	Tamoxifen	10						

Summary

The anticancer activity of hexane and ethanolic extract of all the three plant were screened by MTT assay. The result obtained from the anticancer activity shows that among the hexane extract tested, the hexane extract of *Azima tetracantha* demonstrated the proliferative efficacy in HeLa cell line. And among the compound tested quercetin isolated from *Azima tetracantha* has most promising proliferative efficacy against HeLa cell line than the other two compounds tested.

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