INVITRO ANTICANCER ACTIVITY OF ETHANOLIC EXTRACT OF PENTATROPIS CAPENSIS (L.f.) Bullock AGAINST HeLa CELL LINES

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Abstract

Cancer is the third leading cause of death worldwide , claiming over 6 million lives every year preceded by cardiovascular and infectious diseases. Plants have a long history of use in the treatment of cancer. Drug discovery from plants is a multi – disciplinary approach which combines various botanical, ethnobotanical, phytochemical, biological and chemical separation techniques. Over 50% of the drugs in clinical trials for antitumor activity were isolated from natural source. The assessment of the cytotoxicity of ethanol extract of *Pentatropis capensis* was based on the data from MTT assay. Our present study observed that the ethanol extract of *Pentatropis capensis* inhibited the proliferation of human cell line(HeLa). From the results obtained, it is apparent that the extract from *Pentatropis capensis* was active in inhibiting in vitro cell proliferation. The number of colonies decreased significantly in a concentration dependent manner, suggesting that *Pentatropis capensis* suppressed the regeneration potential of cancer cells in higher concentrations.

Keywords: *Pentatropis capensis*, cisplatin, hela cell lines, MTT assay.

Introduction

Pentatropis capensis is a twining herb of the family Apocynaceae. The local name of the species is „Uppilankodi". The crude extract of the plant have been used as a traditional medicine for the treatment of various diseases such as antifungal, anti rheumatic, anti-inflammatory and analgesic and also for treating cold and diarrhoea. Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health It is a generic term for a group of more than 100 diseases that can affect any part of the body. Although there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Accordingly, several new strategies are being developed to control and treat cancer. One such approach could be a combination of an effective phytochemical with chemotherapeutic agents, which when combined would enhance efficacy while reducing toxicity to normal tissues**.** The interest in natural products research has resulted in discovery of more efficient drugs for cancer treatment (Calixto, 2000; Rates, 2001and Phillipson, 2001).

Materials and Methods

Collection and authentication of plant material

The fresh and young aerial parts of *Pentatropis capensis* (Apocynaceae) were collected from in and around Coimbatore, Tamil nadu, India. The collected plant materials were identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical survey of India, Southern circle Coimbatore, Tamil Nadu.

Sample preparation

Coarse powder from the shade dried aerial plant parts of *Pentatropis capensis* (500 g) was exhaustively extracted using Soxhlet apparatus with absolute ethanol (78.5°C). The extract was dried (free of solvent) using a vacuum evaporator for condensation. The extract thus obtained was stored in refrigerator and used for In vitro antioxidant activities and anticancer activities.

***In vitro* Anticancer studies**

The human cervical adenocarcinoma cell line (HeLa) was obtained from National Centre for cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following extract addition the plates were incubated for an additional 48 h at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using GraphPad Prism software.

Results

***In vitro* Anti cancer studies**

The assessment of the cytotoxicity of ethanol extract of *Pentatropis capensis* was based on the data from MTT assay. This method was employed for determining the anti proliferative activity of ethanol extract of *Pentatropis capensis* in culture. In this present study, the HeLa cell lines were incubated for 48 hours with increasing doses of

ethanol extract of *Pentatropis capensis*, ranging from 18.75 µg/ml to 300 µg/ml. MTT dye reduction assay was done to assess the proliferative activity. Results obtained are shown in Plate 1 and Table 1. Decrease in proliferation of treated cells was observed when compared to the untreated controls. Ethanol extract of *Pentatropis capensis* induced apoptosis in HeLa cell lines in a dose dependent manner with a mean IC_{50} (concentration of drug required to reduce the cell viability to 50%) value of 312.1µg/ml and R^2 value was 0.9878.

Table -1.
Cytotoxic activity of ethanolic extract of *Pentatropis capensis* on HeLa cell lines

Plant Extract	Concentration µg/ml	HeLa		IC_{50}	R^2
		Absorbance	% inhibition		
Pentatropis capensis	Control	0.337333		312.1	0.9878
	18.75	0.323	0.978474		
	37.5	0.281667	5.18591		
	75	0.25	17.31898		
	150	0.173333	26.61448		
	300	0.340667	49.11937		

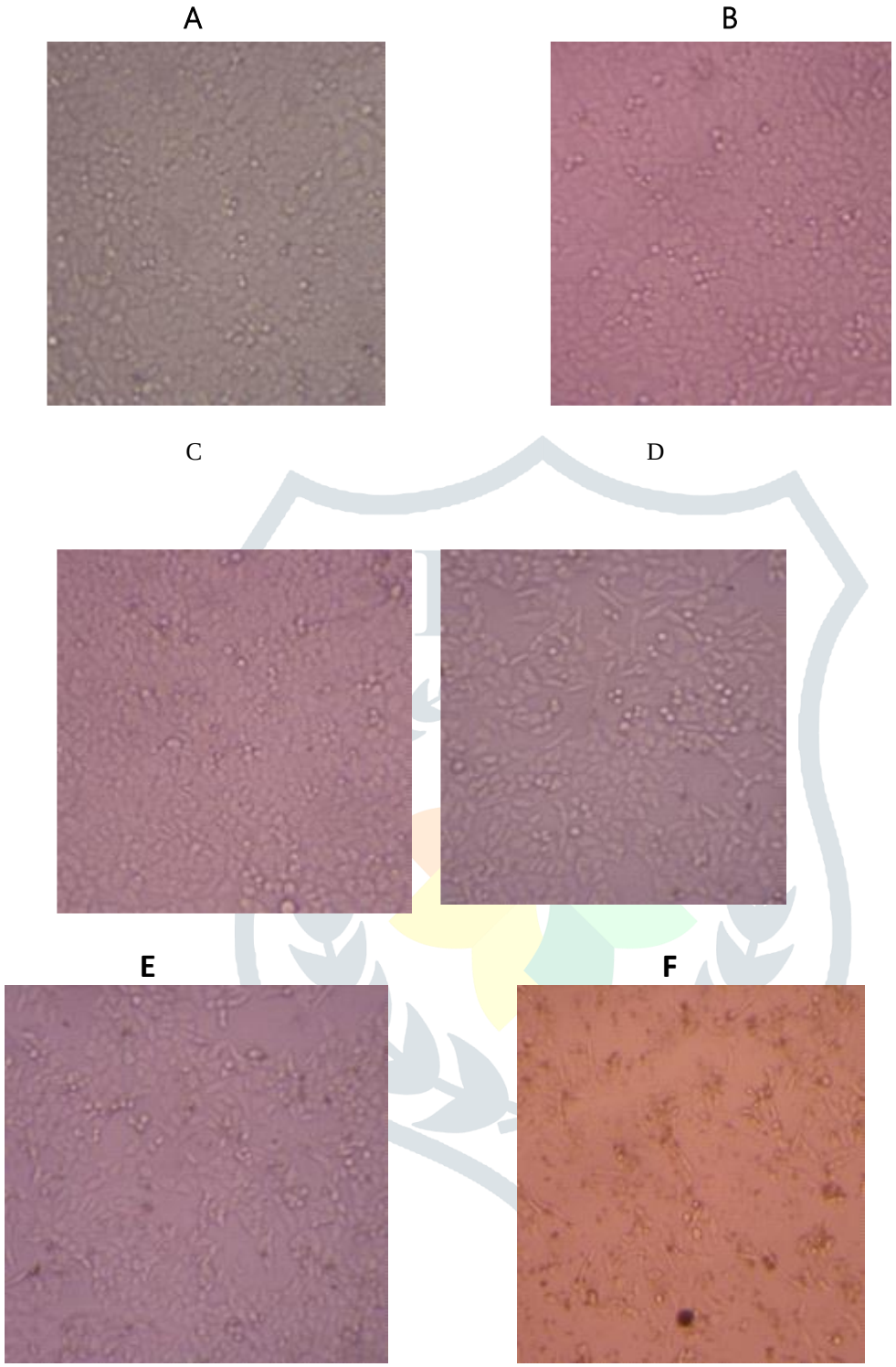
Discussion

In vitro Anticancer studies

Since cervical cancer is the second most common cancer among women worldwide, it continues to be a serious health problem. Cisplatin is often used in combination with 5-fluorouracil and considered as the gold standard treatment for women with locally advanced cancer of the cervix. These chemotherapeutic drugs destroy cancer cells by interfering the cell division and growth. The affected cells become damaged and eventually die. However, apart from affecting the cancer cells, these chemotherapeutic drugs also affect normal cells cause severe side effects such as nausea and vomiting, unusual bleeding, fever, chills, body aches, sores in the mouth and throat, chest pain, problems with vision, seizure, jaundice, cardiovascular side effects including myocardial infarction, deep vein thrombosis, blood pressure, myasthenic syndrome, cortical blindness, nephrotoxicity, strokes and leukoencephalopathy.

PLATE- 1

Effect of ethanolic extract of *Pentatropis capensis* against HeLa cell lines



a- Control
b- Cytotoxic effect of ethanolic extract of *Pentatropis capensis* on HeLa cell lines at 18.75 µg/ml concentration
c- Cytotoxic effect of ethanolic extract of *Pentatropis capensis* on HeLa cell lines at 37.5 µg/ml concentration
d- Cytotoxic effect of ethanolic extract of *Pentatropis capensis* on HeLa cell lines at 75 µg/ml concentration
e- Cytotoxic effect of ethanolic extract of *Pentatropis capensis* on HeLa cell lines at 150 µg/ml concentration
f- Cytotoxic effect of ethanolic extract of *Pentatropis capensis* on HeLa cell lines at 300 µg/ml concentration

Established cancer drug, cisplatin treatment demonstrated inhibitory effect on HeLa cells multiplication at lower concentration but shown toxicity at higher concentration and longer treatment duration (Majumdar, 2001). The use of natural compounds extracted from fruits, vegetables, oil seeds and herbs as an antioxidant and functional foods has become a global trend recently (Wang *et al*., 1997).

Therefore, the need to discover an effective, novel and scientifically reliable natural anti carcinogenic compound is urgent. Natural products provide a fertile ground for seeking out treatments with fewer side effects and equal or better results (Subhuti, 1999). Our present study observed that the ethanol extract of *Pentatropis capensis* inhibited the proliferation of human cancer cell line (HeLa). The extract displayed dose dependent antiproliferative cytotoxicity against the cell line tested. From the results obtained, it is apparent that the extract from *Pentatropis capensis* was active in inhibiting *in vitro* cell proliferation. Moderate cytotoxicity by ethanol extract was observed against HeLa human cancer cell line. The number of colonies decreased significantly in a concentration-dependent manner, suggesting that *Pentatropis capensis* suppressed the regeneration potential of cancer cells in higher concentrations. The results of this study showed a moderate activity of plant extract against the tested cell line, though the IC_{50} values are not lower than the dose recommended by the protocols of the National Cancer Institute of USA (Geron *et al*., 1972) (Table-1). Thus, further *in vivo* study is warranted to confirm its cytotoxicity.

Plant derived natural products such as flavonoids, terpenes, alkaloids and so on have received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and cancer chemoprotective effects (Maicon., 2008). The phytochemical work carried by Umachigi *et al*. (2008) revealed that ethanolic extract of the galls of *Quercus infectoria* contains high amount of tannins, gallic acid, ellagic acid, syringic acid, sitosterol and amenotoflavone, implied that tannin is one of the active compounds which may be responsible for the antiproliferative activity. Several antioxidants in plants have been suggested to contribute to the anti carcinogenic effects and other phytochemicals such as flavanols have been able to inhibit cancer cell proliferation *in vitro* (Scalbert *et al*., 2005). It was reported by Fellows (1992) that ethanol extract has more polar compounds as compared to other solvents. Percentage of inhibition shown by ethanolic extracts were moderately significant which may be due to the terpenoids (Akbar and Malik, 2001), presence of flavonoids (Ferguson *et al*., 2004) and alkaloids (Washed *et al*., 2011). Our results are in agreement with these findings. Phytochemical analysis of the ethanol extract of *Pentatropis capensis* showed the presence of tannins (26.20±1.44) and lower levels of flavonoids (11.28±0.05) and thus justifying the moderate cytotoxicity of the tested plant. The antioxidant studies of ethanol extract of *Pentatropis capensis* also showed that it had low antioxidant activity (Table 1). This fact may be the reason for its moderate antiproliferative activity. This study suggests that *Pentatropis capensis* is moderately effective as an anticancer agent and have a chemotherapeutic potential on cervical cancer cells at higher doses with IC_{50} value of 312.1µg/ml. Plant extract has more than one active compound with different nature and solubility in different solvents. The results obtained from the phytochemical analysis and cytotoxic activity revealed that further investigations may lead to the development of safe and potent anticancer agents from *Pentatropis capensis.*

Conlusion

The present investigation the assessment of cytotoxicity of ethanolic extract of *Pentatropis capensis* by MTT assay revealed moderate activity of plant extract against the tested HeLa cell lines. The presence of different bio active compounds are the reason for its anticancer activity. The outcome of the present study encourages to carry out further studies to be extended for other cell lines and invivo cytotoxicity investigation and required to identify anticancer activity.This also enabled to go further into the molecular level to identify different compounds present in the plant source.

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