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Research Article

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Anti proliferative effect of Herbo mineral formulation Pancha Pashana Chendhuram in cervical cancer cells

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Abstract

There are many old traditional systems flourishing in the world, one among them is Siddha system of medicine. Pancha Pashana Chendhuram is a herbo mineral formulation in Siddha system of medicine which is used to treat various types of cancers. Though it is used traditionally for the treatment of cancers it is necessary to prove scientifically for the benefit of mankind. For that purpose, Pancha Pashana Chendhuram was tested for its Anti-proliferative effect on cervical cancer cell line, HeLa. Results show that moderate activity of compounds with LD 50 value of 92ug/ml which can be considered significant. This study reveals that Pancha Pashana Chendhuram has Anti-cancer activity and can be better alternative medicine for cervical cancer patients.

Keywords: Pancha Pashana Chendhuram, Anti-cancer activity.

Introduction

Carcinoma of the cervix continues to be the most common genital cancer encountered in clinical practice in India. The universal application of pap smears in western communities has led to a drastic decline in the number of invasive cancers of the cervix and a higher detection of preinvasive lesions. Five lakh new cases are reported annually world over. In India alone, 130,000 new cases occur with the death toll of 70,000 every year. Cancer of the cervix accounts for 15% of all cancers in females.[1]It is mostly caused by a genital Human Papillomavirus (HPV). In developing country like India poor economic condition and low standard of living are the principal reason for susceptibility to cervical cancer. Treatment generally includes surgery, radiation therapy, chemotherapy or in combination. Though modern treatment methods yield good results, Cancer patients who receive radiation therapy are also prone to develop secondary tumors because of the DNA damage induced by radiations. Kopraj et al, 2002 have reported in a study about the DNA damage induced by different combinations of radiation therapy and these may induce a secondary tumour in cancer patients.[2] So, there is a need for alternate treatment method to radiation therapy, for which traditional medicines can be a better solution. Ayurveda and Siddha are the two major traditional medicinal systems that originate in India. Traditional medicines have their source from plants, metals and minerals which contains active ingredients and have potency to cure many diseases including cancer.

Materials and Methods

Details regarding sample

Pancha Pashana Chendhuram is a classic Siddha herbo-mineral formulation mentioned in Sikitcharathinadeepam.[3]

Table 1. Ingredients of Pancha Pashana Chendhuram

GROUP I	GROUP II
Purified Thalagam (Arsenic trisulphidium)	Vettrilai *(Acalypa indica)*
Purified Lingam (Red sulphide of mercury)	Vettrilai*(Piper betle)*
Purified Rasam (Hydrargyrum)	Paruthi *(Gossypium hirsutum)*
Purified Gandhakam (Sulphur)	Vellarukan *(Calotropiesprocera)*
Purified Vellaipashanam (White arsenic)	Thulasi*(Ocimum sanctum)*
Purified Manosilai (Red orpiment)	Uthamni *(Pergulariadaemia)*
Purified Kaantham (Magnetic oxide of iron)	Poduthalai *(Phyla nodiflora)*

Drug collection

All the ingredients were obtained from country drug shop, Ramasamychetti, Parrys Chennai, Tamilnadu, India.

Identification and Authentication

All the raw drugs were identified and authenticated at Central Research Institute (CRI), Chennai and Botany department, Govt Siddha Medical College, Arumbakkam, Chennai.

Preparation of the drug

All the group I drugs are grinded with group II herbal juices for 12 hours and made into small poultices (Villai). Then it is to be dried in the sun shade. The dried poultice is covered with *Piper betle* leaf. The covered poultices are placed in mud plate and closed with same size of another mud plate and is sealed with 7 layers of mud pasted cloth. Then the contents are ignited with Mild flame for 12 hours. Then the collected Chendhuram is again subjected to heat for 8 hours. The end product is grinded as a fine powder and preserved in an air tight container.

Details regarding experiment

In vitro antiproliferative effect determination by MTT assay

HeLa (cervical cancer cell line) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen).

The cellline was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extracts and compound stock:

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Antiproliferative Evaluation:

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Antiproliferative Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Antiproliferative Assay by MTT Method:

15mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well,

then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize theformazan crystals.

Results

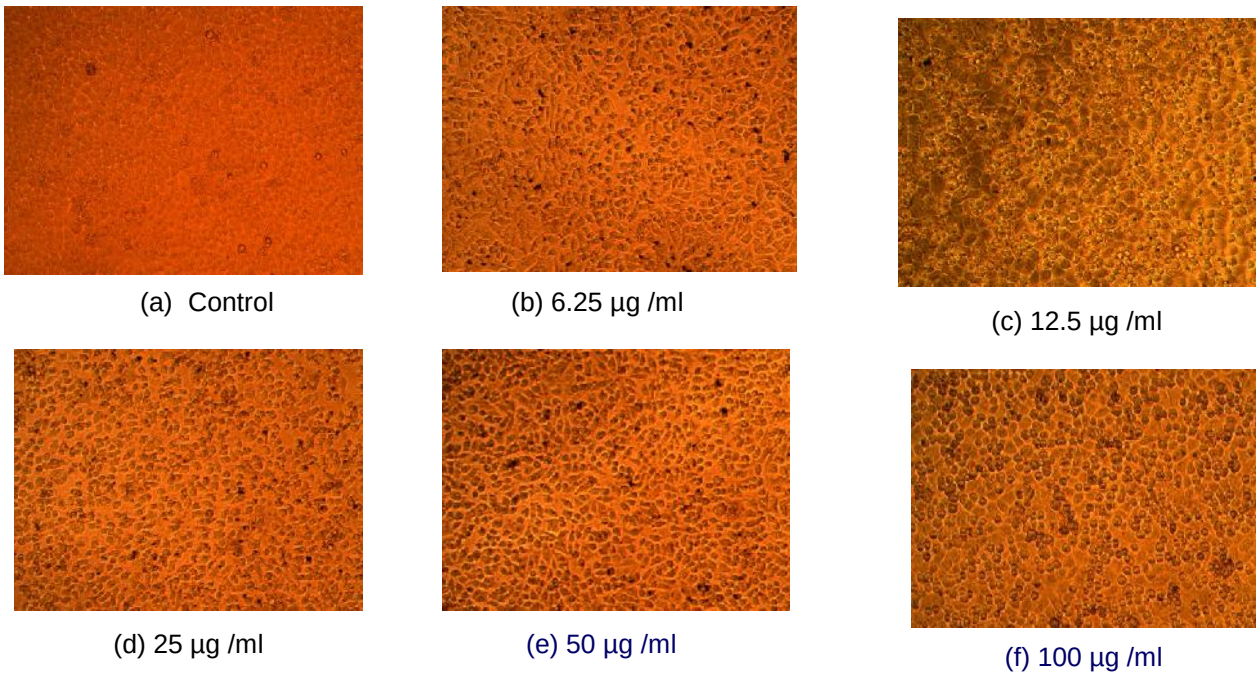
The absorbance values were measured by using microplate reader at a wavelength of 570 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of control group}}$$

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage Viability
Control	1.7645	
6.25	1.605	90.96061
12.5	1.5606	88.44432
25	1.2282	69.60612
50	1.1281	63.93313
100	0.8766	49.6798

LD 50 value –91.6955**µg/ml**



(a) Control (b) 6.25 µg /ml (c) 12.5 µg /ml

(d) 25 µg /ml (e) 50 µg /ml (f) 100 µg /ml

Figure 1: Anticancer effect of Pancha Pashana Chendhuram in HeLa cell line

Discussion

Since ancient times, plant-based formulations have been practiced as remedies against diverse ailments[4]. Shifting of peoples towards traditional medicines is increasing in recent days. Siddha and Ayurveda are the two major traditional systems of medicine in India. Pancha Pashana Chendhuram is one among the numerous cancer drug formulations mentioned in Siddha system. The outcome of MTT assay shows that there is moderate activity of compounds with LD_{50} values of 92ug/ml which can be considered significant. The major goals in cancer treatment and also the emphasis of the present study are inhibition of proliferation and induction of apoptosis of cancer cells[5]. Phase contrast analysis of cell morphology also shows round cells with membrane damage and blebbing which can be considered as indicators of apoptosis (programmed cell death), a desirable property of anti cancer drugs.

Conclusion

The results acquired through MTT assay reveals that the Siddha formulation, Pancha Pashana Chendhuram has significant anticancer activity. There was considerable inhibition of proliferation and the LD_{50} value was 92ug/ml. Further, all the ingredients of PanchaPashanaChendhuram need to be screened against the HeLa cell line separately to confirm the activity.

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