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Invitro Anticancer Activities of Siddha Herbomineral Formulation Panchapadana Chenduram

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ABSTRACT:

Background Various in vivo and in vitro studies have found the effects of herbo minerals in treating cancers and cervical cancer being the 4th commonly occurring cancer in women. The combined anticancer, anti-oxidant effects of herbs, minerals and heavy metals provide us a scope for treating cancer patients effectively. In this study we studied the effect of Panchapadana Chenduram on HeLa (cervical adenocarcinoma) cell lines at different concentrations.
Objective To observe the level of anticancer and antiproliferation capacity of Panchapadana Chenduram on HeLa (cervical adenocarcinoma) cell lines.
Materials and methods The HeLa (cervical adenocarcinoma) cell lines were treated with different concentration of 10, 50,100,150 and 200µg/ml. The viability of the cells is checked, percentage growth inhibition using MTT assay.
Results At varying concentrations, significant changes were observed in the cell viability, antiproliferative capacity of the test drug. High percentage of cell death was observed at the concentration of 200µg/ml 35.65 ± 1.606 % and IC_{50} value of the sample was found to be 276.1 ± 22.12 µg/ml evidencing that the test drug PPM possess convincing anti-cancer activity.
Conclusion The results of the current study point towards the usage of Panchapadana Chenduram as an efficient and safe chemotherapeutic agent in future, with more evidences required on HeLa (cervical adenocarcinoma) cell lines to treat cervical cancer.

1. Introduction

Cancer is defined as abnormalities in cell regulatory systems resulted from mutation, cell division in an uncontrolled manner and loss of control normal cell function. This continual unregulated proliferation of cancer cells extends its spread to surrounding tissues, organs and throughout the body leading to its dysfunction and abnormal activity.[1] The normal principal regulatory genes where mutation takes place are the growth inhibiting tumor suppressor genes, growth promoting proto-oncogenes, genes that regulate cell death or apoptosis and the genes involved in DNA repair. In traditional medicine metastatic cancer is studied as Oduvippuruthi characterized by Red and white rashes, lump in the body, pain in the bone, ulceration in the body. In literature Mega Kattikal were classified into many types based on the size of the tumor and its location.[2]

Among the types of cancer cervical is 4th common cancer among women worldwide with persistent HPV infection being the major cause in developing countries. Every year, there are more than 500,000 new cases of cervical cancer and approximately 250,000 deaths due to cervical cancer worldwide. Differing in the growth pattern or cytological morphology they are classified as squamous cell carcinomas and invasive adenocarcinomas.[3]The treatment options for invasive cancer include surgical resection (modified radical hysterectomy) in early stages. However most patients would require adjuvant treatment with chemotherapy and radiation followed by surgery, depending on the disease high risk pathology.[4]

In the traditional siddha medicine, the combined antioxidant, anti-inflammatory and anticancer activity of several herbs and minerals together tested in vivo and in vitro, are used successfully in treating cancer as a primary therapy or adjuvant therapy since a long time. The most commonly used herbo-mineral formulations are Chenduram (sulfide form of mineral/ or metals), Parpam (oxide form of mineral/or metals), Chunnam (a caustic oxide preparation) and Pathangam (a product of sublimation). There exists a preconception on combining heavy

metals and minerals with herbal formulations concerning the safety and toxicity aspects. Siddha literature evidently shows, the usage of minerals and metals in their nontoxic form because of their individual therapeutic values. The toxic micro or macro particles in heavy metals are converted to nontoxic Nano particles by the kind of herbal extraction process they go through. The nanonature of metals and plant bioactive compounds enhance the usefulness of herbo-mineral drugs to treat various cancers. Combining metals and minerals with herbs also increases the shelf life of the formulation from 75 to 100 years and also offer a better efficacy just in smaller doses.[5]

Studies have shown the efficacy of herbominerals such as Chithiramoola kuligai, Gaanthaparpam, Panchakalpa chendhooram, Rasaganthi mezhugu, Vellai seelai, Gandhaga chendooram, Ayaveera chendhooram used as treatment for cancers like prostate cancer, vaginal cancer, breast cancer, mouth cancer, uvular cancer, cancer of genitals, stomach cancer and some herbominerals in all types of cancer including metastatic cancer.[6]

In this current study we analysed the antiproliferative and apoptotic effect of the herbo mineral pancha pasana chendooram on HeLa (cervical adenocarcinoma) cell lines. This herbomineral formulation contains Ocimum sanctum (Thulasi), Acalypha indica (Kuppaimeni), Enicostema axillare (Vellarugu), Piper betle (Vetrilai), Gossypium (Paruthi), Pergularia daemia (Veliparuthi), and Lippia nodiflora (Poduthalai) mixed with minerals such as red sulfide of mercury (Lingam), mercury subchloride (Pooram), white arsenic (Vellai pasanam), arsenic trisulfide (Thalagam), arsenic disulfide (Manosilai), sulfur (Gandagam), and magnetic oxide of iron (Kaantham). This study is carried out with the evidence of anticancer properties in the herbs and with minimal evidence of usage against cancers like breast cancer and prostate cancer.[7]

2. Materials and Methods

Preparation of Test Solutions

10mg/ml concentration of the sample PPM was prepared using DMSO at the concentration of 10, 50,100,150 and 200µg/ml. The diluted sample PPM was transferred to the culture plate.

Hela (Cervical Adenocarcinoma) Cells Culture and Media

HeLa cell lines were procured from NCCS, stock cells were cultured in medium supplemented with DMEM (Dulbecco's Modified Eagle Medium), penicillin (100 IU/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5% CO_2 incubator. [8]

Source of reagents: DMEM, FBS, Pen strip, Trypsin procured from Himedia.

Anti- Proliferation Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 48hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured

using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

$$\text{Survival rate (\%)} = \frac{A_{sample} - A_b}{A_c - A_b} \times 100$$

IC_{50} Value

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve.

MTT Assay

The *in vitro* determinations of anti-proliferative effects of the test formulation have been performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt upon incubation MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. The resulting colored solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.[9, 10]

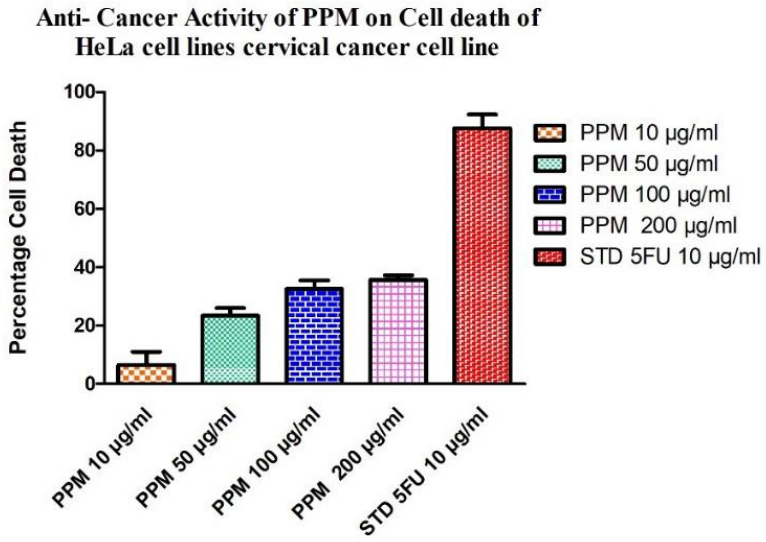
3. Results

The present observations made, with In-vitro anti-cancer evaluation of test drug PPM on the cell death against HeLa (cervical adenocarcinoma) cell line was performed at varying concentration ranges from 10 to 200 µg/ml .The result obtained from the study reveals that the percentage of cell viability of HeLa (cervical adenocarcinoma) cell line decrease with increase in concentration of the test drug PPM. High percentage of cell death was observed at the concentration of 200µg/ml 35.65 ± 1.606 %, followed by this at 100 µg and 50 µg shows 32.54 ± 2.91, 23.37 ± 2.678, similarly at 10 µg/ml it shows 6.46 ± 4.535 % cell death in MTT assay. The corresponding IC_{50} value of the sample was found to be 276.1 ± 22.12 µg/ml (Table-1 & Figure-1). It was concluded from the result of the present study that the test drug PPM possess convincing anti-cancer activity.

Table-1. Effect of Test drug PPM on Cell death of HeLa (cervical adenocarcinoma) cell line

S.No	Concentration in µg/ml	% cell Death
1	10 µg/ml	6.46 ± 4.535
2	50 µg/ml	23.37 ± 2.678
3	100 µg/ml	32.54 ± 2.91
4	200 µg/ml	35.65 ± 1.606
5	STD (5-Fluorouracil 10 µg/ml)	87.58 ± 4.718
	IC 50 Value of PPM	276.1 ± 22.12

Figure-1. Anticancer Activity of PPM on Cell Death of Hela (Cervical Adenocarcinoma) Cell Line



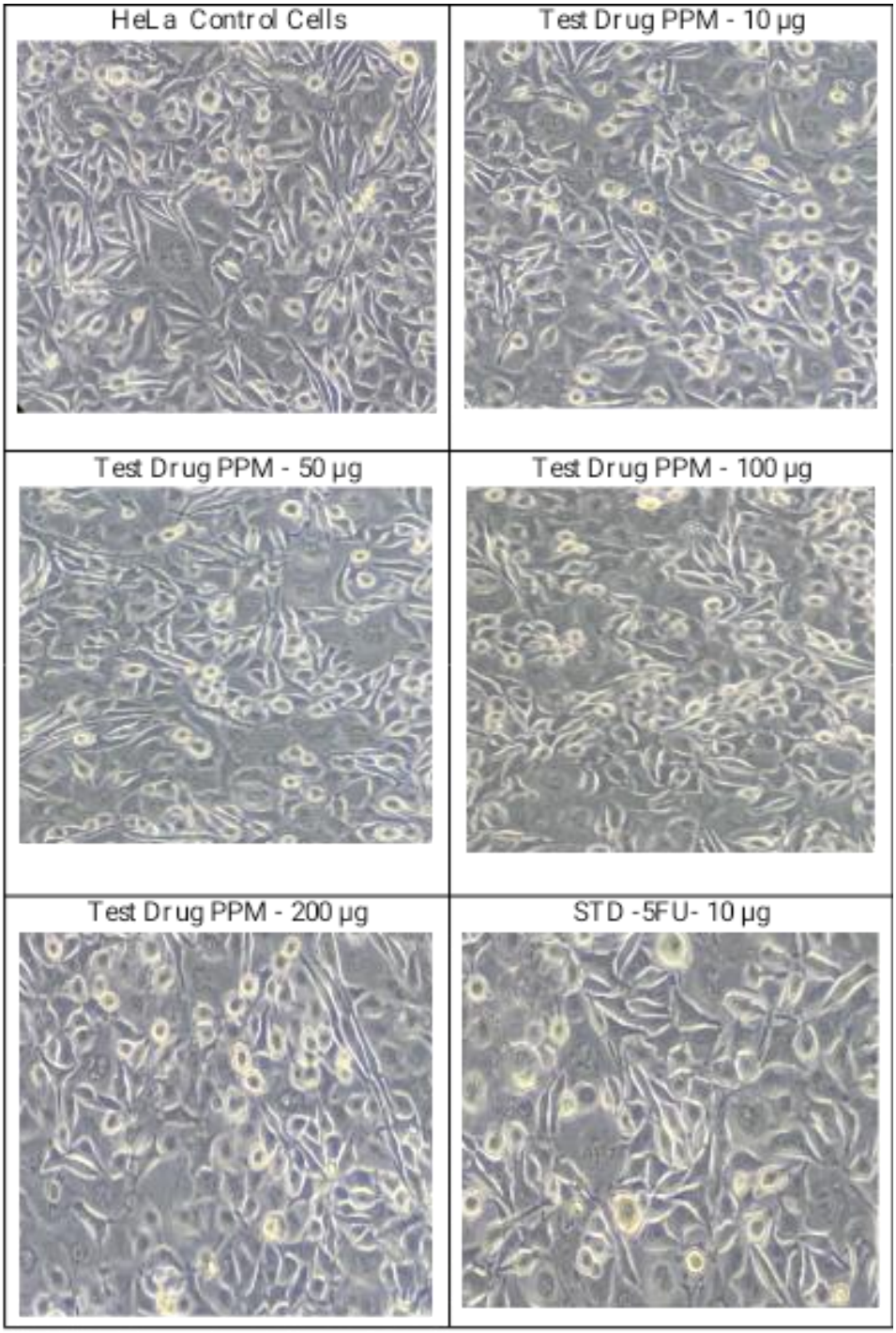


Figure-2. MTT assay of Test drug PPM

4. Discussion

Apoptosis or cell death of the abnormally proliferating cells and restoring the usual metabolism of the cells is the main target in the treatment of cancer. The phytochemicals like alkaloids, taxanes, epipodophyllotoxins and camptothecins are currently being used in the modern anticancer regimen for their anticancer properties. Paclitaxel and docetaxel have very high activity in a spectrum of solid tumours (ovarian, breast, lung, head and neck, gastrooesophageal, bladder, testis, endometrium neoplasms from Taxus buccata. Flavinoids present in Mimosa pudicahas, Glycyrrhiza glabra- Athimathuram exhibited maximum

cytotoxic effect against MCF-7 and Human breast cancer cell line. Another herb Plumbago zeylanica was evidently used in the treatment against liver cancer, spread of breast cancer, fibrosarcoma, malignant ascites and leukaemia. Ocimum gratissimum - Rama Thulasi/ African Basil - significantly alter viability of lung adenocarcinoma A549 cells through a synergy of induction of apoptosis.

Some preclinical studies have evaluated the apoptosis and anti-proliferative effect of herbs andminerals like Surulpattai chooranam against skin melanoma SK-Mel28 cell lines, Gowri Chinthamani Chendhooram (GCC) on HeLa cell lines, rasagenthi lehyam (RL) against prostate cancer PC-3 cells by using MTT assay. Siva Guru Kuligai (SGK) a herbomineral is also tested in vitro for its anticancerous properties. From all such evidences in the literature certain herbominerals are considered being used as a novel complementary and an alternative therapy for patients on chemotherapy and radiation.[2, 11] The time- and dose-dependent inhibition of Chloroform Extract of Rasagenthi Mezhugu against both SiHa and ME-180 cervical cancer cells was determined using MTT assay determining the integrity of mitochondria and viability of the surrounding cells.[12]

The preparation panchapadana chendooram is a purified red colour metallic powder formed obtained by the process of either burning them or frying them or exposing to the sunlight or keeping them in specialized pudas by adding decoctions, cheyaneers, dravagams, etc. The mixture is prepared from the red sulfides of mercury (lingam), magnetic oxide of iron (gaantham), mercury subchloride (pooram), arsenic trisulfide (thalagam), white arsenic (vellai pasanam), arsenic disulfide (manosilai) and sulfur (gandagam).[7,13] One of the main ingredients in the preparation lingam (Cinnabar) is an age old traditional medicine used in siddha for its anti-inflammatory, anti-diarrhoeal, analgesic, antipyretic, anxiolytic properties and also scientifically proved for its anticancerous properties invitro. Hence it is used in most of the preparations like Puttru pathakam, Namachivaya Chenduram, Pancha pashana Chenduram, Kaalamega narayana Chendhooram, Ashta Bairava Chenduram and Panchamuga Chendhuram.[14]

A compound found in Acalypha indica, quercetin is tested invitro against breast cancer cell lines MCF-7 and MDA-MB-231 showed drug's impact on DNA integrity, cellular damage and displayed increased apoptosis activity when compared with doxorubicin, a regularly prescribed medicine for cancer.[15] Another study conducted on Ocimum Sanctum showed a significant decrease in the level of intracellular glutathione and increase in lipid peroxides when compared to untreated Sarcoma-180 cells in the hind limb of mice. A significant reduction in the tumor volume was also noted in the same study.[16] Suppression of cell proliferation was observed in the leaves of Enicostemma littorale by measuring the antitumor initiating potential, expression pattern of apoptotic (p53, Bcl-2 and Bcl-2 associated X-protein), cell-proliferative (cyclin D1 and proliferating cell nuclear antigen), and inflammatory (NF-κB and cyclooxygenase-2) markers, invasive (matrix metalloproteinase-2 and 9) markers in 7, 12-dimethylbenz (a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis.[17] Significant changes in the cell morphology leading to cytotoxicity and apoptosis were found when KB- cancer cell lines were treated with Piper betle leaf extract.The MTT assay showed that the percentage viability of the cancer cells decreased with increasing concentrations of the extract.[18] In our study when the percentage of cell viability of HeLa (cervical adenocarcinoma) cell lines were measured at different concentrations of the test drug, greater decrease in the cell viability was found at 200 µg/ml when compared to the standard 5-fluorouracil. With the above evidences of activity of herbs and minerals combined against cancer cells, the present result results in our study add much evidence to the current by measuring the cell viability and inhibitory concentrations of the test drug Panchapadana Chenduram using MTT assay.

5. Conclusion

With the evidences that support the current study findings, Panchapadana Chenduram can be used as an alternative safe and effective treatment option for cervical cancer reducing the disease burden of such highly prevalent conditions in the world. Although much evidence is required to further support the current literature, this study confirms the significant anticancer effects of ppm.

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