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Anticancer Activity of Thambira Chendhuram in A549 Cell Line in Vitro Method

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ABSTRACT

Siddha system is one of the traditional system in south India. The system has peculiar way toclassify the disease and treatment. Medicine formulation was prepared by Plants, metals and minerals. When the disease does not respond to plant preparation after the metals and minerals was used for the treatment process, particularly the chronic anduncured disease were treated with Perumarunthugal, like Parpam,chendhooram, Kattu, Kalangu. Nowadays people struggled with lot of disease especially Cancer is one of the major burden in present situation. Cancer mentioned in Siddha literature, known as Putrunoi, Kazhalai, vippuruthiit was treated by Perumarunthugal. Thambirachendhuram is the one of our best medicine in Siddhatreasurer, the drug was used to treat Cancer in internal viscera's and leprosy. The present study was conducted, to evaluate the anti-cancer efficacy of Thambirachendhuramin lung cancer cell line (A549). A549 cell line was treated with Thambirachendhuramin different concentration in vitro method and the result was noted. The test drug TC on the cell viability againstA549 cancer cell line was performed at varying concentration ranges from 6.25, 12.5, 25, 50 and 100µg/ml and percentage cells viability in MTT assay. The IC50 value was found to be TC-53.7163µg/mL.

KEYWORDS: Siddha, Cancer, chendhuram, A549, Lung cancer, Puttrunoi

I. INTRODUCTION

Cancer is the second most leading cause of death in worldwide.An abnormal growth of cells which tend to proliferate in an uncontrolled way and in some cases it leads to metastasis [1].Lung cancer, also known as lung carcinoma, is a malignant lung tumor characterized by uncontrolled cell in tissues of the lung. This growth can spread beyond the lung by the process of metastasis into nearby tissue or other parts of the body [2[1]. Most cancers that start in the lung, known as primary lung cancers, are carcinomas.1.61 million of new cases reported for lung cancer per year in worldwide, in cancer related mortality lung cancer is the leading cause of death with 1.38 million deaths, approximately 63,000 new lung cancer cases were reported each year in India[3]; it is the commonest cancer and cause of cancer related mortality in men.

The two main types of lung cancer:

1. Small cell lung carcinoma (SCLC)
2. Non small cell lung carcinoma (NSCLC).

Siddha medicine**,** traditional system of healing that originated in South India and is considered to be one of India's oldest systems of medicine. Siddha the term derived from the word "siddhi" [4]. Siddha system of medicine helps to cure disease and to attain the perfection of life. The nature of the system continues service to humanity for more than five thousand years.

The treatment in Siddhabased on the Plants, Metals and Minerals and Animal origin. If this does not prove effective, the judicious use of plants, minerals, and animal products is advised.Siddha medicine has been used for the management of chronic diseases .Its effectiveness in those situations has varied [5].In Siddha literature, Cancer is known as Putru noi, Vippuruthi, Katti, Kazhalai,are found in the diseases that are described by the ancient Siddha texts under different names such us "Puttru Noi", "Vipprudhi","Kandankkirandhi","Kaddi","Kalalai" ,"Raajapilavai","Thurmaangisam","Maamisamakotharam","Sathaiadaippu","Utkaarasoolai" and "Silandhi"[6].

But Cancer patients count increased in day by day and any system of medicine to treat cancer is a challenge without side effects and it only increases the life time of patients. Still there is a need for medicines with fewer side effects and cures the disease initial stages of cancer and prevent the metastasis.

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Herbo metallic compound drug have been used to treat cancer and nowadays it has been great attention to the treat cancer. So the main objective of this study explained about preclinical study of anti cancer activity in cell line model to treat by ThambiraChenduram. ThambiraCenduram is one of the higher order drug and herbo metallic preparation in Siddha system of medicine to treat Cancer and leprosy from " The Pharmacopoeia of Siddha Research Medicines"So this is the time to validate anti cancer activity of ThambiraChenduram in A549 cell line.

II. MATERIALS AND METHOD

2.1 Selection of the trial drug:

The trial drug "**ThambiraChenduram"**is a herbo metallic combination drug select fromSiddhaliterature"The Pharmacopoeia ofSiddha Research" written by Dr.M. Shanmugavelu, page no: 62.

2.2 Collection of raw drug for ThambiraChenduram:

- The raw drug copper plate was bought from Ramasamychetty country shop at Parrys, Chennai.
- Fresh Plant material was collected from around Anna Hospital campus, Arumbakkam, Chennai.

2.3 Identification and authentication:

The raw drug copper plate and fresh plant materials were identified and authenticated by the experts of Gunapadam (Pharmacology) and Botanist at Government Siddha Medical College, Arumbakkam, and Chennai.
The specimen samples of the identified raw drug were preserved in the laboratory of P.G Gunapadam for future references.

2.4 Ingredients:

- Copper Plate
- Adathodaiverpattai (Justiciabeddomei)
- Vennochiverpattai (vitexnegundo)
- Erukkampaal (Calotropisgigantea)

Copper plate was purified as per siddha literature[7] and the plant materials were purified and "ThambiraChenduram" was prepared as per SOP [8].After the completion of this process, the obtained Chenduram was taken and kept in an air tight container.

2.5 Drug Profile:

Drug Name :"ThambiraChenduram"
Dosage : 32.5 Mg – 65 Mg (1/2 To 1 Grain) BD
Adjuvant: Honey
Route of Administration : Enteral
Reference : "The Pharmacopoeia of Siddha Research Medicines"
Publication : G.D. Naidu Charities.
Indications: Carcinoma of the internal viscera and Leprosy.

2.6 Preservation:

Prepared "ThambiraChenduram" was preserved in well stoppered air tight glass containers and labeled as TC ("ThambiraChenduram").

2.7 In vitro anticancer activity of ThambiraChendhuram for non small cell lung cancer determination by MTT assay[9]

A549 (Lung cancer)cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma aldrich, USA).

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin 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 were 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) wasseeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of compound stock:

1mg of sample was weighed and dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer Evaluation:

After 24 hours the growth medium was removed, freshly prepared each compounds in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in

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500µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Non treated control cells were also maintained.

Anticancer Assay by Direct Microscopic observation:

Entire plate was observed after 24 hours of treatment 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.

Anticancer Assay by MTT Method:

Fifteen mg 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 30µl 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(Dimethyl sulphoxide) DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize theformazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm.

The percentage of growth inhibition was calculated using the formula:

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

III. RESULT AND DISCUSSION:

A549(non-small cell lung Carcinoma) cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM were free from any kind of bacterial and fungal contamination.

Table No. 1. Anticancer activity of A549 cell line

Sample Concentration (µg/mL)	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	0.6885	0.6946	0.7119	0.6983	100.00
Sample code: TC					
6.25	0.6738	0.6547	0.6351	0.6545	93.73
12.5	0.5542	0.5863	0.6100	0.5835	83.56
25	0.4697	0.3973	0.4168	0.4279	61.28
50	0.2954	0.2746	0.2878	0.2859	40.95
100	0.1616	0.1761	0.1643	0.1673	23.96

IC50 Value: TC- 53.7163µg/mL (Calculated using ED50 PLUS V1.0 Software)

Interpretation:

In-vitro anti-cancer activity of test drug TC on the cell viability againstA549 cancer cell line was performed at varying concentration ranges from 6.25, 12.5, 25, 50 and 100µg/ml and percentage cells viability at 6.25 µg/ml shows 93.73%, followed by 12.5 µg/ml shows 83.56%, 25 µg/ml shows 61.28%, 50 µg/ml shows 40.95% and 100 µg/ml shows 23.96 %µg/ml Least viability of cell was observed at the concentration of 100µg/ml shows 23.96 % in MTT assay. The percentage of cells viability was determined by calculating the O.D of treated against the control. Reading optical

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density (OD) is performed in a spectrophotometer at a wavelength of 540 nm. The IC50 value was found to be TC- 53.7163µg/mL

Chart No.1. Anticancer activity of A549 cell line

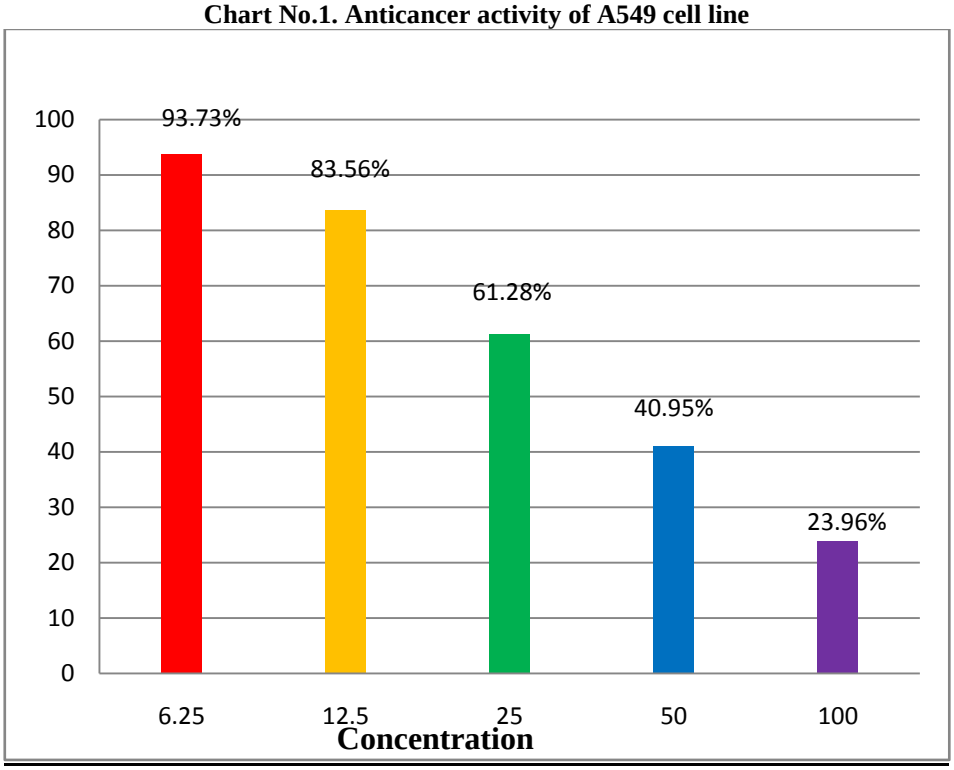


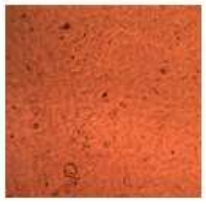
Chart No 1 shows various concentration of TC and % of Inhibition of A549 cells after the treatment. It can be observed by the result of MTT assay that the IC value of TC is 50µg/ml at the concentration decreases the A549 cell viability. It was found that the % growth inhibition increasing with increasing concentration of TC steadily up to 6.25 µg / ml on A549 cell line and that IC value on A549 cell line was TC- 53.7163µg/mL

Lungscancer cell line treated with various concentrations of TC

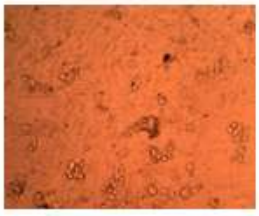
Fig No. 1. Lungscancer cell line treated with various concentrations of TC

Control

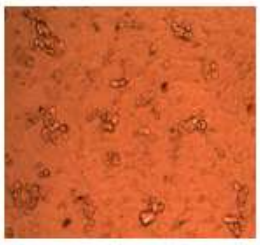
TC treated with 6. 25µg/mL



TC treated with 12.5 µg/mL



TC treated with 25 µg/mL



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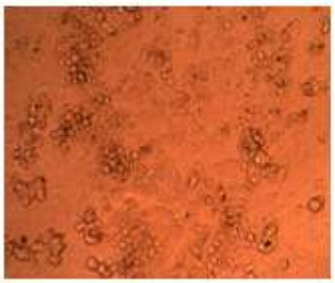
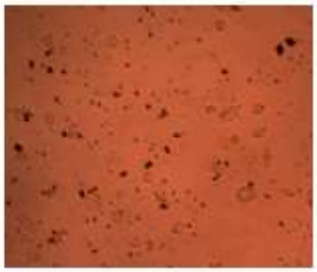
TC treated with 50 µg/mL



TC treated with 100 µg/mL



IV. CONCLUION:

TC at different concentration (6.25-100 µg in 500 µl of 5% DMEM) was administered for 24 hrs. To determine the cytotoxic effect of Siddha formulation TC against A549 cell line. To conclude from this study, being a cytotoxic compound in the drug is one of the prerequisites for an anti-cancer drug and results of this in vitro study on the herbo metallic preparation TC support one of the key Complementary Alternative Medicines in cancer therapy.

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