SIDDHA PAPERS
A PEER REVIEWED ONLINE JOURNAL
ISSN: 0974-2522

Siddha Papers 2018 (13) (2)
RESEARCH ARTICLE
https://www.siddhapapers.com

IN-VITRO ANTICANCER ACTIVITY OF SIDDHA FORMULATION *CHITHIRA PALLATHI MEZHUGU* AGAINST *HELA* CELL LINE

Selvam.M[1], Kingsly.A[2], Essakkypandian.G[3], Antony Duraichi.R[4]

1. P.G Scholar, Department of *Gunapadam*, Govt.Siddha Medical College, Palayamkottai.
2. Head Of The Department of *Gunapadam*,Govt. Siddha Medical College, Palayamkottai.

3, 4 Assistant Lecturer, Grade.II, Department Of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai.

Email: vijayaselvambsms24@gmail.com

ABSTRACT

Siddha system of medicine is a well defined science in the medical world. It is one of the most ancient systems of medicine for curing diseases. The symptoms of cervical carcinoma are blood tinged vaginal discharge, painless menorrhagia, malignancy enlarges. This symptom may be correlated with *karuppaikazhunthuputtru* in our siddha system. Siddhars told a wonderful medicine for *karuppaikazhunthuputtru* namely *Chithira Pallathi Mezhugu*. This traditional preparation is being used for ages without scientific validation. In this current study, the attempt is made to establish the fact of anticancer activity of the drug above. In modern scientific approach, this anticancer study can see through In-vitro *HeLa* cell line method. This cell line method is used for cervical carcinoma. The name *HeLa* cell line was derived from the name of the lady Henrietta Lacks who had affected by the cervical carcinoma at first. This study results found that the % growth inhibition increasing with increasing concentration steadily up to 30.127µg/ml on *HeLa* cell line and IC50 value of this assay. Now overall study evaluate that Siddha Formulation *ChithiraPallathiMezhugu* has potential activity on *HeLa* cell line. So this drug has considerable anticancer activity on cervical carcinoma.

KEY WORDS: *ChithiraPallathiMezhugu* (CPM), *Karuppai Kazhunthu Puttru*, Cervical Carcinoma.

1. INTRODUCTION

Siddhars told about many type of carcinomas with treatment. Cervical Cancer is one among the most life threatening diseases affecting women all over the world. Compared to other cancers, cervical cancer is rare. Cervical cancer represents 0.8% of all new cancer cases in the U.S. In 2017, it is estimated that there will be 12,820 new cases of cervical cancer and an estimated 4,210 people will die of this disease. Cervical carcinoma symptom may be correlated with *KaruppaiKazhunthuPuttru* in our Siddha system. *Siddhars* told about a wonderful medicine for *Karuppai Kalunthu Puttru* namely *ChithiraPallathiMezhugu*(CPM). In this current scientific study was done to evaluate cytotoxicity activity of *ChithiraPallathiMezhugu* against *HeLa*cell line. In modern scientific approach, this anticancer study can see through In-vitro *HeLa* cell line method. This cell line method is used for cervical carcinoma.

2. CHITHIRAPALLATHI MEZHUGU

2.1PREPARATION OF DRUG

INGREDIENTS

1. Purified Marking nut	: 100 No's
2. Purified Sesame	: 175 gm.
3. Purified Mercury	: 35 gm.
4. Purified Sulphur	: 70 gm.
5. Jaggery	: 175 gm.

2.2 PURIFICATION OF DRUGS

1. **MARKING NUT(*Cherangkottai*)**

   The nose like part of marking nut is to be removed and fried with gingely oil.

2. **SESAME (*Ellu*)**

   Take Sesame in a vessel, pour water and kept for few hours then washed and remove the skin layer then dried out.

3. **MERCURY*(Sootham*)**

Mercury	: 35 gm.
Brick powder	: Required quantity
Turmeric powder (*Curcuma longa*)	: Required quantity
Indian Acalypha juice (*Acalypha indica*)	: 1.3 litre

Mercury is triturated with brick powder and turmeric powder for one our respectively and washed with water. Then the mercury is boiled with the juice of Indian Acalypa until it is detoxified.

4. SULPHUR (*Gandhagam*)

Sulphur is placed in an iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts; this mixture is immersed in inclined position in cow milk. This procedure is repeated for 30 times to get purified sulphur. Each time, fresh milk is used.

2.3 PREPARATION PROCESS

Sesame and Jaggery is grinded separately. Then mercury and sulphur are triturated separately and both are mixed with the above ingredients, add *Cherangkottai kuli thylam* (125.5gm) and grinded for one day. When it reaches the waxy consistency, it is stored safely.

2.4 PRESERVATION OF DRUG

It is stored in an air tight glass container.

2.5 ADMINISTRATION OF THE DRUG

Form of the drug	: Wax (*Mezhugu)*
Route	: Enteral (oral)
Dosage	: *Manipunganvithai* (100-200mg) twice a day
Shelf Life	: 5 years
Indications	: CervicalCancer(*Karuppai Kalunthu Puttru*), Penile Cancer *(Linga Puttru)*

3. CELL LINE STUDY

Cell line: Cervical carcinoma cell line *(HeLa*).

Tetrazolium salt assay (Microculturetetrazolium Test or MTT)

3.1 MTT ASSAY

3.1.1 OBJECTIVE

To determine the cytotoxicity of *Chithira Pallathi Mezhugu* (CPM).

3.1.2 PRINCIPLE

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colour water soluble Tetrazolium dye MTT to

Formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble Formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured Spectrophotometrically at 570nm.

3.1.3 MATERIALS

1. Cell lines:*HeLa* cell(From NCCS, Pune)
2. Cell culture medium: DMEM- High Glucose - (#AL111, HiMedia)
3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
4. Fetal Bovine Serum(#RM10432, HiMedia)
5. MTT Reagent (5 mg/ml) (# 4060 HiMedia)
6. DMSO (#PHR1309, Sigma)
7. D-PBS (#TL1006, HiMedia)
8. 96-well plate for culturing the cells (From Corning,USA)
9. T25 flask (# 12556009, BioLite - Thermo)
10. 50 ml centrifuge tubes (# 546043 TORSON)
11. 1.5 ml centrifuge tubes (TORSON)
12. 10 ml serological pipettes (TORSON)
13. 10 to 1000µl tips (TORSON)
14. 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (ELX-800 BioTek)
15. Inverted microscope (Bio link)
16. 37°C incubator with humidified atmosphere of 5% CO_2 (Heal force, China)

3.1.4 ASSAY CONTROLS

- Medium control (medium without cells).
- Negative control (medium with cells but without the experimental drug/compound).
- Positive control (medium with cells and with 5% DMSO).

  Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-Tocopherol, Dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

3.2 PROCEDURE FOR DETERMINING CELL CYTOTOXICITY

3.2.1 CELL SEEDING

1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 12 hours.

2. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet).

3. Incubate the plate for 24 hours at 37°C in a 5% CO_2 atmosphere.

4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.

5. Wrap the plate with aluminium foil to avoid exposure to light.

6. Return the plates to the incubator and incubate for 3 hours.

7. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)

8. Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO).

9. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT Formazan crystals especially in dense cultures.

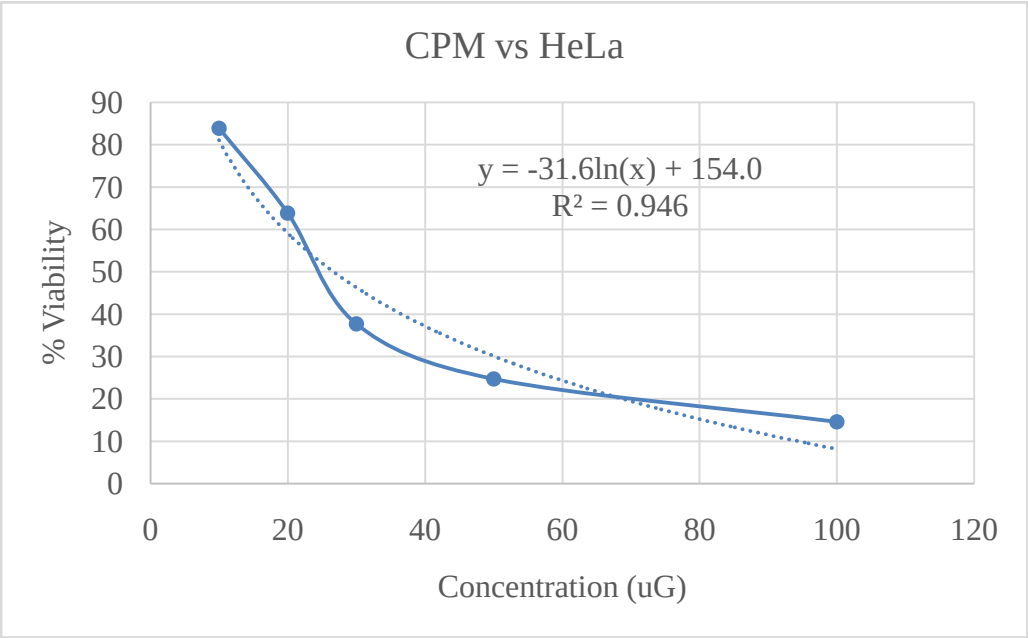
10. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.

11.The Ic50 value was determined by using linear regression equation i.e. Y=Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

3.2.2 DETERMINATION OF CYTOTOXICITY BY MTT ASSAY

Concentration Unit: uG	**BLANK**	**UN TREATED**	**CPT (25uM)**	**10**	**20**	**30**	**50**	**100**
Reading 1	0.011	0.604	0.342	0.515	0.395	0.244	0.161	0.092
Reading 2	0.013	0.613	0.337	0.51	0.391	0.23	0.158	0.106
Mean	0.012	0.6085	0.3395	0.5125	0.393	0.237	0.1595	0.099
Mean OD-Mean B		0.5965	0.3275	0.5005	0.381	0.225	0.1475	0.087
STANDARD DEVIATION		0.0063639	0.00353	0.0035	0.0028	0.009899	0.002121	0.0098
STANDARD ERROR		0.0045006	0.002502	0.0025	0.002	0.007001	0.0015	0.0071
Viability %		100	54.9036	83.906	63.873	37.72003	24.72758	14.585

IC_{50} VALUE= 30.127uG



4. RESULTS

It was found that the percentage of growth inhibition increasing with increasing concentration steadily up to 30.127µg/ml on *HeLa* cell line and IC_{50} value of this assay. Now overall study evaluate that Siddha Formulation *Chithira Pallathi Mezhugu* has potential activity on *HeLa* cell line. Highest percentage of inhibition (83.90612%) obtained at the concentration of 10µg/ml. The results suggested that the *Chithira Pallathi Mezhugu* significantly inhibited the proliferation of human cervical cancer HeLa cells.

5. DISCUSSION

In this research study reveals that CPM has a potent anticancer activity. It also proved that *Chithira Pallathi Mezhugu* (CPM) has been very effective in treating cancer by Kosayi Anupoga Vaithiya Piramma Ragasiyam. Further evaluation are needed to isolate the active ingredient.

6. CONCULSION

Management of cancer with a holistic approach, devoid of any side effects is now the major challenge to the medical system. This work highlights *Chithira Pallathi Mezhugu* as novel anticancer agent which provides a basis for the traditional use of it and proves that it could provide a cost effective and holistic remedy, without any side effects.

REFERENCES

1. S.P.Ramachandran, *Kosayi Anupoga Vaithiya Piramma Ragasiyam*, Part 1,Thamarai Noolagam, Chennai,Pg. 251, 252.
2. Dr.R.Thiyagarajan.L.I.M, *GunapadamThathu-JeevaVagupu*, Published by Directorate of Indian Medicine and Homeopathy, Chennai, 2nd– 3rd part, Pg.244,245, 304, 305.
3. Dr.C.S.Murugesa Muthaliyar, Siddha Materia Medica(medicinal plant division),Published by Directorate of Indian medicine and homeopathy, Chennai, First edition;2008,Pg. 162, 236,490.
4. T.V. Sambasivampillai, Dictionary based on Indian Medicine science, published by Directorate of Indian Medicine and Homeopathy, Chennai, India.
5. Terry Sharrer. *HeLa* Herself. The Scientist 2006; 20: 22.
6. Freshney RI. Culture of animal cells: A manual of basic technique. 5th edition, NY,Wiley-Liss, Inc., 1987. Pg. 200, 201.