

In-Vitro Anticancer Activity of Siddha Formulation Chanda Maarutha Chendooram against *Hela* Cell Line.

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ABSTRACT

Siddha system is one of the natural way of treating systems. Siddhars used a lot of natural herbal, mineral and metal for treating many diseases. Nowadays, this siddha system of medicine is used in southern part of India only. Siddhars told many type of carcinomas with treatment. Carcinoma is one of the life threatening diseases in the world. In recent years many of the women are affected from carcinoma due to life style changes, some bad habits (like cigarette smoking, alcohol) and multi sexual partners etc. The symptoms of cervical carcinoma are blood tinged vaginal discharge, painless metrogia, malignancy enlarges. This symptom may be correlated with *karuppai kalunthu puttru* in our siddha system. Siddhars told that the wonderful medicine for *karuppai kalunthu puttru* name as *Chanda Maarutha Chendooram*(CMC). CMC is made from *pancha sootham* (except mercury) and sulfur. 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 Invitro 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 0.0196 µg/ml on *HeLa* cell line and IC50 value of this assay was 0.264 mg/ml. Now overall study evaluate that Siddha Formulation Chanda Maarutha Chendooram has potential activity on *HeLa* cell line. So these drug has considerable anticancer activity on cervical carcinoma.

Key words

Chanda Maarutha Chendooram, CMC, *karuppai kalunthu puttru*, cervical carcinoma.

INTRODUCTION

Siddha system is one of the earliest traditional medicine system in the world which treats not only the body but also the mind and the soul. The word *Siddha* has its originates from the Tamil word *Siddhi* which means "an object to be attained" or "perfection" or "heavenly bliss". "*Siddhargal*" or *Siddhars* were the premier scholars of this system in ancient times. *Siddhars*, mainly hailing from the Tamil Nadu, laid the foundation for *Siddha* system of medicine. *Siddhars* told that many type of carcinomas with treatment. Carcinoma is one of the life threatening diseases in the world. In recent years many of the women are affected from cervical carcinoma due to life style changes, some bad habits (like cigarette smoking, alcohol)and multi sexual partners etc. The symptoms of cervical carcinoma are blood tinged vaginal discharge, painless metrorrhagia, malignancy enlarges. In India cervical carcinoma is the second most common carcinoma among women and also the second most common carcinoma among women between 15 and 44 years of age. In south india, carcinoma Cervix is the most common carcinoma among females. The incidence of CA Cervix in chennai is 99 per 1,00,000. Cervical carcinoma symptom may be correlated with *karuppai kalunthu puttru* in our siddha system. *Siddhars* told that the wonderful medicine for *karuppai kalunthu puttru* name as *Chanda Maarutha Chendooram*(CMC).. In this current scientific study was done to evaluate cytotoxicity activity of *Chanda Maarutha Chendooram* against HeLa line. In modern scientific approach, this anticancer study can see through Invitro 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.

PREPARATION OF DRUG

Ingredients of the drug are,

1. Purified *Rasakarpooram* 10 *Varaagan* (35g)
(calomel)

2. Purified *Lingam* 8 *Varaagan* (28g)

(Natural cinnabar)

3. Purified *Veeram* 2 *Varaagan* (7g)

(Hydrargyrum perchloride)

4. Purified *Gandhagam* 2 *Varaagan* (7g)

(Sulphur)

5. Purified *Rasa chendooram* 2 *Varaagan* (7g)

(Red sulphide of mercury)

6. Raw rice 2½ *Palam* (87½g)

7. Bottle gour 2 ½ *Palam* (87½g)

8. Hens' egg white q/s

PURIFICATION OF RAW DRUGS :

1) Rasa karpooram – (Calomel) : The poultice made of betel leaf (*Piper betle*) and pepper (*Piper nigrum*) each 8.75 gm is taken and dissolved in 1.3 litre of water. Calomel 35gm tied with a cloth and immersed in the liquid from the cross bar without touching the bottom of the vessels and heated. After the water is reduced to 3/4 of its volume, the calomel is taken out, washed with water and dried to get it in purified form.

2) Lingam - (Natural Cinnabar) : When the crude form of red sulphide of mercury is soaked for one day in mother's milk and lemon juice respectively, it becomes purified.

3) Veeram - (Hydrargyrum perchloride) :

Perchloride of mercury 35gm is consolidated with pepper decoction (*piper nigrum*) for 6 hours. Then it is buried within the pepper poultice. Sodium chloride 650gm and camphor 35gm are mixed well and kept in a mud pot in which the above poultice is buried and burnt for some hours with low intensity fire to get the purified form of perchloride of mercury.

4) Gandhagam – (Sulphur) :

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's milk. This procedure is repeated for 30 times to get purified sulphur. Each time, fresh milk is to be used.

5. Rasa Chendooram – (red sulphide of mercury) :

It is kept soaked in lemon juice for 24 hours. It is then washed and dried to get purified form.

PREPARATION OF THE DRUG :

All the ingredients are finely powdered first and then triturated with hens' egg white for 2 saamam (5 hours). Then, cakes are made from the mixture and dried. Take a new pot; add some water to it and boil it, till bubbles appear. Then, add the cakes to it. The cakes are removed from water, after they are cooked. Then, they are dried. Raw rice and bottle gourd are taken in a pot and boiled till bubbles appear. Then the cakes are added. After 2 minutes, the cakes are taken back; washed with cold water and dried in sunlight. Then, it is powdered and stored safely.



Chanda Maarutha Chendooram

PRESERVATION OF DRUG :

It is stored in an air tight glass container.

The drug was prepared by standard operating procedure.

Administration of the drug :

Form of the drug : chendooram

Route : enteral (oral)

Dosage : ½ to 1 kundri (65 to 130gm).

Adjuvants : PalmJaggery, Butter, Driedgingerpaste, Panchadeepakini
Illagham,Thirikadugullagham,ThiripalaIllagham,Castor oil.

Shelf Life : 75 years

INDICATIONS:

6 types of *puttru* (cancers), 13 types of sannai, Athisaaram (Diarrhoea), Powthiram (Fistula), 80 types of vatha diseases, 40 types of pittha diseases, 18 types of soolai, paarisa vaayu, 6 types of paandu (Anaemia), Vidhai vatham, Anda vatham (hydrocoele), Oodhu kaamaalai, Aadi alaru sannai, Muha vatham (Facial palsy), 18 types of kuttam, 8 types of gunmam, Kakkai vali (Epilepsy), Soothaka vali (Dysmenorrhoea), Kanu vayu, kanu vatham, pun (Ulcer), Kiranthi, Vedi soolai, soolai erivu, Pakkavali, Kandamaalai, Thamaraga vayu, Paambu kadi (Snake bite), pooraan kadi (Centipede bite), Naaikadi nanju (Rabies), Elik kadiv nanju (Rat bite), Vippuruthi, Suram (Fever), Karappan (Eczema), Karappan related Madhumegam (Karrappan related Diabetes mellitus).

CELL LINE STUDY:

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

Table 1: Details of cell lines.

Cell line	Morphology	Origin	species	Ploidy	charecteristics	Supplier
Hela	Epethelial	Cervix	Human	Aneuploid	G6PD type A	NCCS,pune

Cell proliferation kit: MTT (Roche applied sciences, Cat. No. 11465 007 001)

Tetrazollum salt assay (Microculture tetrazolium Test or MTT)

Principle

The MTT assay utilizes a color reaction as a measure of viable cells. The assay is dependent on the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazolium salt to a blue formazan product by the mitochondrial dehydrogenase of viable cells/ metabolically active cells. The intensity of blue colored formazan produced is directly proportional to the cell viability.

Procedure

The cells from a particular cell line when in log phase of growth are trypsinized, counted in a haemocytometer and adjusted to appropriate density in a suitable medium and then inoculated in different 96-well plates. The cells are treated with various concentrations (in replicate) of drugs for specified duration (usually 1 to 4 days) after which MTT dye is added in each well and plates are incubated at 37 °C for 4 hours in a CO₂ incubator. The plates are then taken out of incubator and dark blue colored formazan crystals are thoroughly dissolved in DMSO at room temperature. The plates are then read on an ELISA reader at 570nm. The percent cell viability with respect to control is calculated using the formula

$$\% \text{ cell viability} = \frac{\text{OD of treated cells}}{\text{OD of control cells}} \times 100$$

The DMSO as a solvent rapidly solubilizes the serum as well as formazan and use of spectrophotometric grade DMSO gives stable background absorbance levels. Other solvents like isopropanol, propanol, hexane and nethylformamide though used, do not solubilize serum at concentrations exceeding 0.0625 percent.

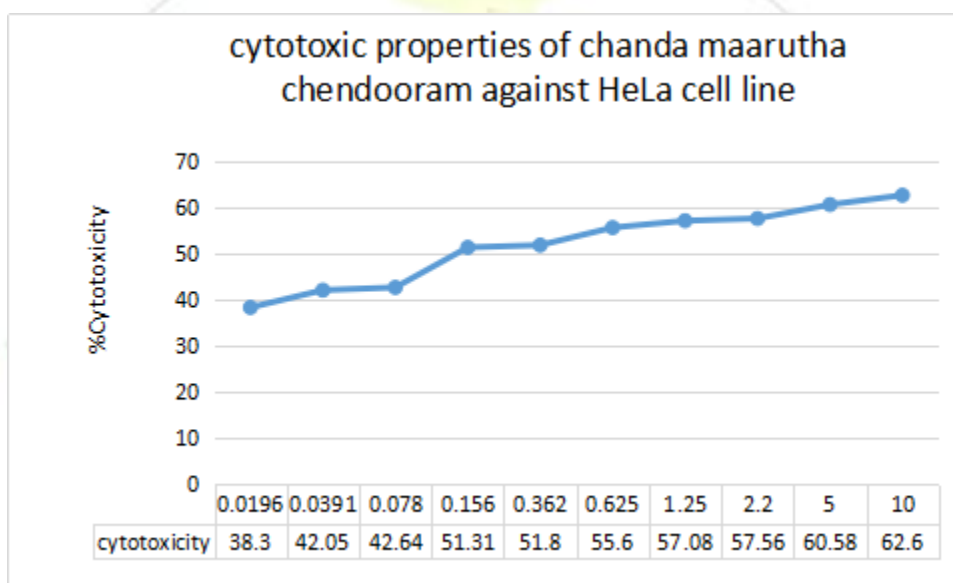
Determination of Cytotoxicity by MTT assay

Table 2: Determination of cytotoxicity by MTT assay.

Siddha formulation	Concentration µg/ml	Hela Absorbance	% Inhibition
Chanda	10	1.520	62.60
Maarutha	5	1.558	60.58
Chendooram	2.2	1.618	57.56

	1.25	1.626	57.08
	0.625	1.660	55.60
	0.362	1.738	51.80
	0.156	1.746	48.31
	0.078	1.920	42.64
	0.0391	1.932	42.05
	0.0196	2.004	38.30

As per MTT assay shows significant effect on *HeLa* cell.



IC50 – inhibitory concentration (concentration of drug required to inhibit 50% cell growth)

RESULTS

It was found that the percentage of growth inhibition increasing with increasing concentration steadily up to 0.0196 $\mu\text{g/ml}$ on *HeLa* cell line and IC50 value of this assay was 0.264mg/ml. Now overall study evaluate that Siddha Formulation *Chanda Maarutha Chendooram* has potential activity on *HeLa* cell line. Highest percentage of inhibition (62.6%) obtained at the concentration of 10 $\mu\text{g/ml}$. The results suggested that the *Chanda Maarutha Chendooram* significantly inhibited the proliferation of human cervical cancer HeLa cells.

DISCUSSION

In *Hakkim P. Mohamed Abdullah Sahib* text book, it is said that *Chanda Maarutha Chendooram* is very effective in treating cancer. In this current scientific study reveals that CMC has a potent anticancer activity. Further evaluation are needed to isolate the active ingredient.

CONCLUSION

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

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Abbreviations

CMC	- <i>Chanda Maarutha Chendooram</i>
CA	- Cancer
DMSO	- Dimethyl sulfoxide
OD	- On Dose
MTT	- Microculture Tetrazolium Test

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