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IN VITRO EVALUATION OF ANTICANCER ACTIVITY OF KUKKILATHI CHOORNAM – A SIDDHA MEDICINE AGAINST HeLa CELL LINES

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ABSTRACT

Free radicals are highly reactive chemicals that have the potential to harm cells. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions. Cancer is one of the dreadful diseases and the population affected is also increasing day by day. Even the well advanced modern science struggles to save cancer patients from the complications. Siddha medicine is one of the most ancient medical systems of India. Siddha is the mother medicine of ancient Tamils/Dravidians of peninsular South India. The word Siddha means established truth. In Siddha literatures 4448 types of diseases were mentioned. Siddhars reported cancer as 'Putru noi' and have mentioned many potent drugs which are having anti oxidant properties. Among them, KUKKILATHI CHOORNAM (KC) a classical drug having anticancer potential is mentioned in Siddha Literature. MTT assay for KC was done on HeLa cell lines. At the end of this preliminary study, KC was found as a potent medicine on human cancer cells. Further studies with this medicine will pave right way towards safe and potent anticancer medicine.

KEYWORDS: Anticancer activity, Kukkilathi Choornam, HeLa cell lines, Siddha medicine.

INTRODUCTION

Cancer is considered to be one of the leading causes of morbidity and mortality worldwide. The global burden of cancer continues to increase largely because of the changes in life style of peoples. It is believed that cancer risk can be reduced by avoiding tobacco, limiting alcohol intake, limiting UV exposure from the sun and tanning beds and maintaining a healthy diet, level of fitness and seeking regular medical care. Siddha System is one such type of older medical system which was practiced by the peoples in South India especially in Tamil Nadu. It not only deals with the treatment of diseases but also relies greatly on living a healthy life. The term cancer is explained in Siddha system as *Putru noi*.

Cervical cancer is found to be one of the most common types of cancer in women. In recent years, the use of herbal medicines in cancer treatment has received increasing attention due to their various phyto-metabolic contents with multiple biological activities. Hence, it is a need to develop safe and effective treatment method for the prevention and treatment of cervical cancer.

So from the Siddha system of medicine, One of the Siddha herbo mineral formulations *Kukkilathi Choornam* is mentioned in Siddha text Anubhoga Vaidhta Navaneetham – Part VIII. It is a simple herbo mineral preparation. Hence this study was attempted to validate anticancer activity of Siddha herbo mineral formulation through MTT assay on HeLa cells, the first immortal human cancer cell lines.

MATERIALS AND METHODS

Preparation of trial drug

The raw drugs were bought from authorized country raw drug shop at Parrys corner in Chennai, Tamilnadu. All the raw drugs were identified and authenticated by the pharmacological experts from Department of Gunapadam, National Institute of Siddha, Chennai.

INGREDIENTS

The herbo mineral siddha formulation *Kukkilathi Choornam* (KC) is a combination of one mineral and six herbal drugs.

TAMIL NAME	BOTANICAL NAME
Purified Kukkil	Shorea robusta
Purified Parangi sakai	Smilax china
Purified Gandhagam	Sulphur
Kandathippili	Root of Piper longum
Arisithippili	Piper longum
Vetpalai Arisi	Wrightia tinctoria
Vaaividangam	Embelia ribes

PROCEDURE

The ingredients were purified as per Siddha literatures. The drug *Kukkil* was purified by boiling it in coconut water, dried and powdered. The *Sulphur* was purified by melting it in an iron utensil containing cow's butter and poured into cow's milk, filtered and dried. The *Parangi sakai* was boiled in cow's milk, dried and powdered. The drugs *Kandathippili* (Root of Piper longum), *Arisithippili* (Piper longum), *Vetpalai arisi* (Wrightia tinctoria) and *Vaaividangam* (Embelia ribes) were fried in a dry pan and powdered. The powdered drugs were mixed and made into a fine powder. Finally, Vasthirakaayam (filtering with cloth) was done to ensure the finess of Kukkilathi Choornam.

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CELL LINE AND CULTURE

A subculture of HeLa cells in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FCS was added. The cells suspended in the medium by gentle passage with the pipette and the cells homogenized.

SEEDING OF CELLS

One mL of the homogenized cell suspension was added to each well of a 24 well culture plate along with different concentration of powder extract (Choornam) (0 to 300 µg/mL) and incubated at 37°C in a humidified CO_2 incubator with 5% CO2. After 48 hrs incubation the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells cytotoxicity assay was carried out.

CYTOTOXICITY ASSAY

The assay was carried out using (3- (4, 5-dimethyl thiazol-2yl) -2, 5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan production is

directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. After 48 h incubation the wells were added with MTT and left for 3 h in room temperature. All wells were removed the content using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals, absorbance's were read in Lark LIPR-9608 micro plate reader at 540 nm (Mosman et al 1983).The % cell viability was calculated using the following formula:

$$\% \text{ cell viability} = \frac{\text{A540 of treated cells X 100}}{\text{A540 of control cells}}$$

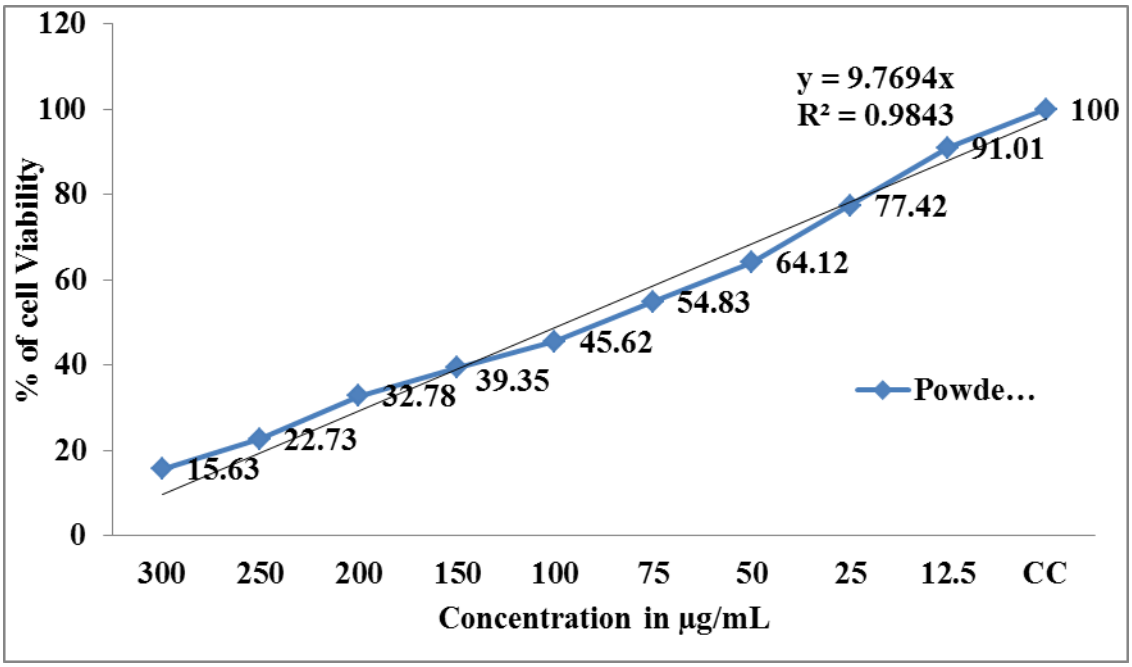
Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

INFERENCE AND OBSERVATION

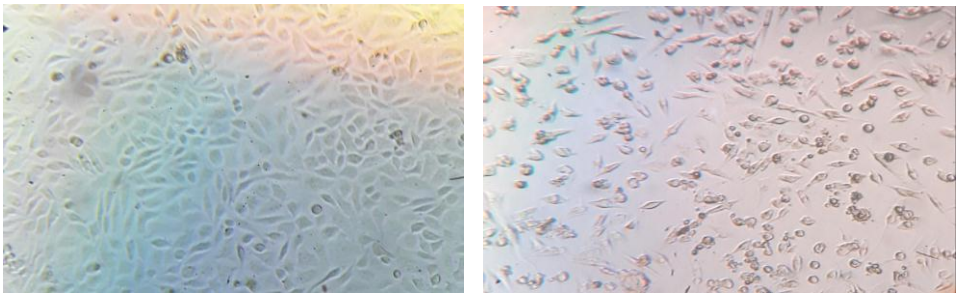
The growth inhibitory nature of Kukkilathi Choornam against HeLa cell lines with different concentrations were studied. When the medicine increased in its concentration, there was an increase in cell growth inhibition. The 50% 0f inhibitory concentration (IC_{50}) of drug value was obtained at 133.169µg/ml. With this concentration it inhibits cell growth effectively. The percentage of cell viability to Kukkilathi Choornam in different concentration is displayed in Table 1. The anticancer activity of Kukkilathi Choornam was plotted in graph 2. The images of HeLa cell lines treated with various concentration of Kukkilathi Choornam are shown in figure 3.

Table 1: Anticancer effect of Sample (KC) on HeLa Cell line at various concentration.

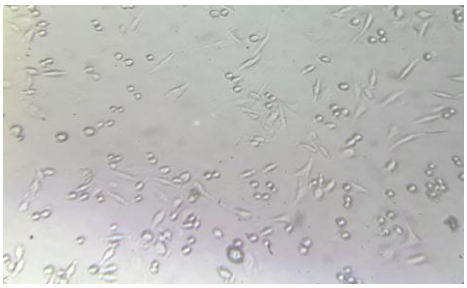
SAMPLE CONCENTRATION(µg/ml)	% CELL VIABILITY
0	100.00
12.5	91.01
25	77.42
50	64.12
75	54.83
100	45.62
150	39.35
200	32.78
250	22.73
300	15.63
IC 50	133.169



Graph 2: Anticancer activity of Powder extract against the HeLa Cell lines.



CONTROL IC_{50} 150 µg/mL



Conc. 300 µg/mL

RESULT AND DISCUSSION

The result obtained from the in vitro studies with HeLa cell lines reveals that the Siddha Herbo Mineral formulation *Kukkilathi Choornam* has potent anti cancer activity and it can be used in the management of cervical cancer affecting majority of women population. Further detailed studies have to be conducted to validate the medicine for global acceptance.

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