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In-Vitro Anti- Cancer Evaluation of Siddha formulation Sathakuppai Chooranam (SKC) against HeLa (cervical adenocarcinoma) cell line by MTT assay

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Abstract

Cancer is clinically characterized by uncontrolled cell proliferation with gene level deficit in controlling the cell multiplication and tissue growth. Alarming statistic suggested that now cervical cancer become the fourth most commonly diagnosed cancer in females worldwide, with 5,28,000 new cases and 2,66,000 deaths annually. In most of the clinical cases symptoms of cervical cancer deliberated at the very lateral stage. In the preset scenario radiation therapy is the most commonly suggested therapeutic measure for treating lateral stage cervical cancer. Increased adverse events towards conventional chemotherapy and repeated desensitization of tumor cells towards radiation most often delays the therapeutic benefits, this grabs the attention of the researcher in the field of cancer biology to explorate the alternate drug of choice which may devoid of side effects. Siddha drug Sathakuppai Chooranam (SKC) is indicated for treating amenorrhea and ovulation induction as per the standard siddha literature. Present study aimed at exploring the anti-cancer property of this novel formulation using HeLa cervical cancer immortal cell line by MTT assay. Activity performed in cell line at varying concentration ranges from 10 to 200 µg/ml. Results of the present study indicates that Least viability of cell was observed at the concentration of 200µg/ml shows 38.64 ± 3.014 %, followed by this at 100µg/ml and 50 µg/ml shows 55.16 ± 3.748, 67.1 ± 3.411 % similarly 10 µg/ml shows 94.59 ± 3.5% cell viability in MTT assay. The corresponding IC50 value was found to be 141.8 ± 7.386 µg/ml. It was concluded from the result of the present study that the formulation SKC possess promising anti-cancer activity.

Key words: Siddha drug, Sathakuppai Chooranam, Anti-cancer activity, MTT assay, Cell viability.

1. INTRODUCTION

Cervical cancer becomes a major global burden and of course considered still one of the most common causes of all cancer deaths in women, especially in developing countries [1]. Viral induction in particular to Human papillomaviruses (HPVs) can disturbs the basic cellular mechanism of growth control [2] and activate the PI3K/AKT/mTOR signaling [3]. It's an common concern as of like other type of cancers cervical cancer will not show any signs or related symptoms during early disease development [4]. Primary symptoms begin with vaginal discharge or bleeding associated with severe back pain [5]. Therefore, most patients who notice symptoms typically have later stages of tumor development that have frequently progressed too far for curative treatment.

Conventional anti-cancer agents offer various potential adverse effects and most of the patients undergo chemotherapy are at high risk of developing anemia, bone marrow depression, neuro degeneration, stress, depression etc. Hence identifying a novel formulation from herbal origin may upsurge the scope of Indian system of traditional medicine [6].

Increased focus on phytopharmaceuticals have attained greater height in recent days, as many number of active pharmaceutical ingredients has been translated to the commercial perspective. Preparations or formulations made of medicinal herbs are right drugs of choice since each herb consist of many number of biologically active phytocomponents, that tend to possess multiple pharmacological action. Most often these components belong to category of secondary metabolites like alkaloids, flavonoids, phenols, glycosides etc [7,8]. It was evident that several anticancer agents are of derived from the herbal sources. Alkaloids like Vinblastine and vincristine are derived isolated phytotherapeutics from the plant Catharanthus roseus which served as gold standard therapy since years [9].

Siddha therapy relies on holistic approach on curing diseases and disorders as narrated by the holy physicians called siddhars. Single and polyherbal preparations are rendering rejuvenation activity by altering the disturbed cell physiology in this context siddha formulation may tend to reverse the cell condition either by upholding the enzymatic activity by mediating the biochemical process. Sathakuppai Chooranam (SKC) is indicated for treating amenorrhea and ovulation induction as per the standard siddha literature. Present study aimed at exploring the anti-cancer property of this novel formulation using HeLa cervical cancer immortal cell line by MTT assay.

2. MATERIALS AND METHODS

2.1. Cell Culture and Maintenance

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), streptomycin (100 µg/ml) in a humidified

atmosphere of 5% CO_2 at 37°C 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 was checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5% CO_2 incubator. Source of reagents: DMEM, FBS, Pen strip, Trypsin procured from Himedia.

2.2.Cytotoxic evaluation (3-[4,5-dimethythiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay)

For anti-proliferative studies, serial dilutions of test formulation (10, 50, 100 and 200 µg/ml) were prepared using DMSO. The monolayer cell culture was trypsinized after successive passage 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 37°C 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 37°C 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 [10,11].

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

3. RESULTS

3.1. Effect of Sathakuppai Chooranam (SKC) against HeLa cell line

Result analysis of the present study have clearly shown that the siddha formulation Sathakuppai Chooranam (SKC) possess significant anti-cancer property in the tested HeLa cell line. Further the result obtained from the study reveals that the percentage of cell viability of HeLa cell line viability decrease with increase in concentration of the test drug SKC. Least viability of cell was observed at the concentration of 200µg/ml shows 38.64 ± 3.014 %, followed by this at 100µg/ml and 50 µg/ml shows 55.16 ± 3.748, 67.1 ± 3.411 % similarly 10 µg/ml shows 94.59 ± 3.5% cell viability in MTT assay. The corresponding IC50 value was found to be 141.8 ± 7.386 µg/ml. As shown in table 1 and figure 1 & 2.

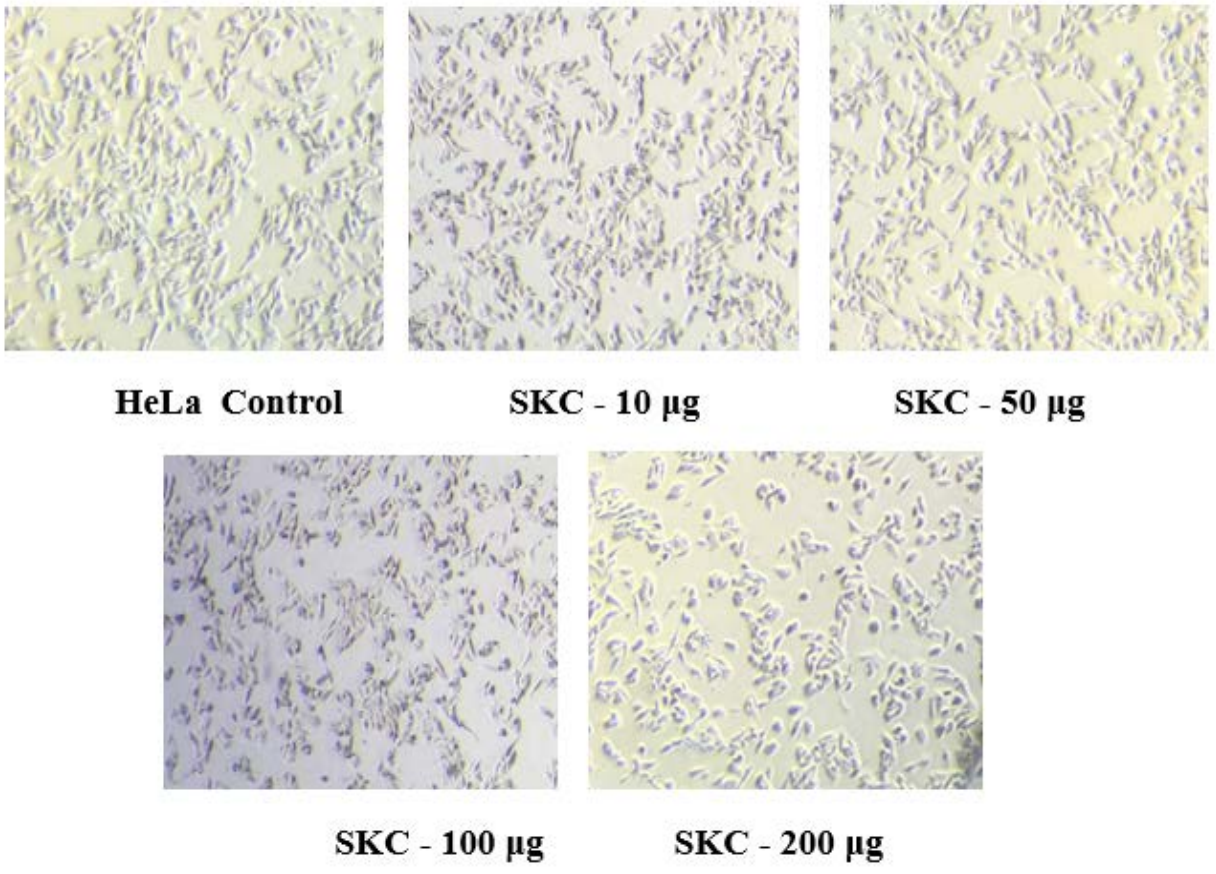

Figure 1: HeLa cell line Incubated with different concentration of test drug SKC

Table 1: Effect of Test drug SKC on Cell death of HeLa (cervical adenocarcinoma) cell line

S.No	Concentration in µg/ml	% cell Death
1	10	5.405 ± 3.5
2	50	32.9 ± 3.411
3	100	44.84 ± 3.748
5	200	61.36 ± 3.014

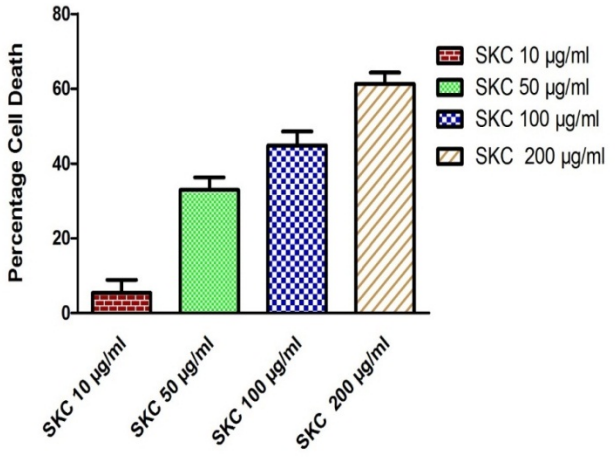

Figure 2: Percentage inhibition of cell viability in HeLa cell line by SKC

4. DISCUSSION

Cancer occurs primary due to environmental carcinogenic moieties that tend to induce the mutation at multiple level [12]. Sustained mitosis the lineage of mutation exchange spread across and finally become uncontrolled proliferation and tumor formation in the specified organ or region [13]. WHO showcase the cancer as one the dreadful disease with the causes of 7.6 million deaths globally. Alarmingly in prosperous nations, around 20% or one in five individuals will die of cancer.In general, predominant anti-cancer drugs may exert its cytotoxicity by optimizing the cell cycle to the condition of apoptosis. Further it was evident through recent studies that phytocomponents like phenols exerts cytotoxic effect mainly through induction of apoptosis [14-16].

MTT assay is one of the gold standard method for the validation of cell viability and to predict the cytotoxic potential of the test drugs. MTT assay works behind the principle of calorimetry technique. Upon incubation the metabolic enzyme present in the active viable cell converts the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in to a formazan dye. Which makes the difference between actively differentiating live cells with that of the dead one. Hence the conversion of yellow colored substrate in to a pinkish red colored product will be quantified upon subsequent wash and incubation for improving the precision in the trial [17,18].

In some cases, the active cell proliferation may be halted by triggering the production of reactive oxygen species [19]. Certainly other way of exerting the anti-cancer mechanism is by regularizing the level of nitric oxide [20]. Other common mechanism that drives the anti-cancer property is inhibition of vascular formation (angiogenesis) thereby nutritious supply to the dividing tumor cells may be arrested. Whereas most of the time researcher have concluded that potential active components present in the formulation claims versatile mechanism of action that precipitate the condition of cell division and halt the disease progression. Accumulation of LDH in the medium may be utilized for ascertain the level of cell damage and DNA fragmentation. Present study reveals that the percentage of cell viability of HeLa celline line viability decrease with increase in concentration of the test drug SKC. Least viability of cell was observed at the concentration of 200µg/ml shows 38.64 ± 3.014 %, followed by this at 100µg/ml and 50 µg/ml shows 55.16 ± 3.748, 67.1 ± 3.411 % similarly 10 µg/ml shows 94.59 ± 3.5% cell viability in MTT assay.

The half maximal inhibitory concentration (IC_{50}) is an important measure of predicating the potency of the formulation or active entity in inhibiting biochemical or metabolic function [21]. Normal quantification of IC_{50} of a drug determined by regular dose-response curve, that the concentration required for the inhibiting the 50 % of the cell population. Lower the value higher will be the potency [22]. From the results of the present study it was observed that IC_{50} value of the test drug SKC was found to be 141.8 ± 7.386 µg/ml based on sigmoid dose response curve.

5. CONCLUSION

Cervical cancer becomes greater cause of death in women of both developed and developing countries. India is rich in cultural and herbal biodiversity are tend to serve newer therapeutic agents from the herbal origin. Siddha system of medicine pioneers the cancer therapy by its hallmark formulations. Siddha drugs are usually safe and devoid of considerable side effect as dealt with the conventional anti-cancer agents. From the results of the present study it was observed that siddha formulation sathakuppai chooranam possess significant anti-cancer activity in the tested HeLa cell line. Further In-vivo studies needs to be carried out to validate the efficacy of the drug before subjecting the same for clinical application in humans.

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